Physics of Cell Fate Decisions 2024

Monday 13th

14:45 – 15:25

Supracellular organization of morphogenesis: epigenetics beyond the cell

Amy Shyer

Rockefeller University, USA

In recent decades, much progress has been made in understanding how genes within cells contribute to organ-specific fates or disease phenotypes. However, it is becoming more widely acknowledged that increasing understanding at the molecular scale has not been sufficient to fully grasp how tissues comprised of thousands of cells generate their structures. To address this gap, my lab centers its studies on the behavior of cell collectives in vertebrate tissues. Using novel collective cell behavioral assays and the skin as a model, we find that emergent biophysical properties arise at the 'supra'cellular scale during organ development. Such emergent properties then serve to shape the skin. Our findings indicate that epigenetic processes beyond the cell scale can organize morphogenesis in vertebrate tissues. Finally, uncovering such epigenetic processes has allowed us to provide an account of morphogen function that re-envisions canonically accepted roles of these chemical cues.

15:25 – 15:50

Time-lapse intravital imaging unveils proliferative heterogeneity and correlated cell fate decisions in mouse interfollicular epidermis

Joel Hochstetter, Dan Engelman (ULB Brussels), Anais Baudin (ULB Brussels), Cedric Blanpain (ULB Brussels), Benjamin Simons (Cambridge); Affiliation: Department of Applied Mathematics and Theoretical Physics and the Gurdon Institute, University of Cambridge

University of Cambridge

In the maintenance of skin interfollicular epidermis, the identity and fate behaviour of the stem cell population remains in debate. In some studies, it is argued that a single equipotent progenitor population captures the main features of the dynamics, whereas others support the idea of multiple discrete proliferative compartments. To challenge the competing one and two progenitor cell models, we perform long-term time lapse imaging of mouse ear-skin epidermis tracing clones for up to 6 months. We compare both 'zero-dimensional' models from the literature, with a novel spatial Voronoi model that incorporates mechanics, allowing a more realistic picture of homeostatic clonal dynamics in squamous tissues. We find that one progenitor models are insufficient to capture fine-grained features of static and temporal clonal dynamics, even when realistic division time distributions and sister correlations are considered. However, we find that a model with a stem cell and transit amplifying progenitor (TA) population can capture correlations in both static clonal distributions and transitions between states in our data. In this picture, clones comprise pairs of stem and TA cells, with highly coordinated decision timing. Our data also illuminates significant biological variability in clonal dynamics between male and female mice, which can be explained within the

framework of our two-progenitor model. These results pave the way for future studies to unravel the molecular mechanisms underpinning cell identity and the feedback mechanisms regulating cell fate decisions of the multiple progenitor populations in the skin epidermis and other squamous epithelial tissues.

15:50 – 16:30

Bayesian inference of chromatin looping dynamics from live-cell measurements

Christoph Zechner

MPI of Molecular Cell Biology and Genetics, Germany

Recent live-cell microscopy techniques allow the simultaneous tracking of distal genomic elements, providing unprecedented ways to study chromatin dynamics and gene regulation. However, drawing robust conclusions from such data is statistically challenging due to substantial technical noise, intrinsic fluctuations and limited time-resolution. I will present recent progress we have made in addressing some of these challenges; specifically, we developed a new statistical method to quantify CTCF/cohesin-mediated chromatin looping dynamics from two-point live-cell imaging experiments. The method combines a simple polymer model with a Bayesian filtering approach to infer loop lifetimes and frequencies. Its application to experimental data revealed that chromatin loops are surprisingly rare (~5% looped fraction) and short-lived (~20mins loop lifetime). I will discuss potential implications of these findings and outline future challenges.

16:30 – 16:55

Cell geometry controls neural induction in early ascidian embryogenesis

Rossana Bettoni, Sophie de Buyl, Géraldine Williaume, Cathy Sirour, Clare Hudson, Hitoyoshi Yasuo, Aleksandra Walczak and Geneviève Dupont; Affiliation: Université libre de Bruxelles

Université libre de Bruxelles

During embryonic development cells adopt different identities with high spatial and temporal precision. How cell fate specification can be controlled in such a reproducible way is a fundamental question in developmental biology. In ascidians, a group of invertebrate chordates, geometry plays a key role in achieving this control. We used mathematical modeling to demonstrate that geometry dictates the neural-epidermal cell fate decision in the 32-cell stage ascidian embryo by a two-step process involving first the modulation of ERK signaling and second, the expression of the neural marker gene, Otx. We developed a mathematical model based on ordinary differential equations to describe signal transduction by the ERK pathway that is stimulated by FGF and attenuated by ephrin, and ERK-mediated control of Otx gene expression. Considering the measured area of cell surface contacts with FGF- or ephrin-expressing cells as inputs, the solutions of the model reproduce the experimental observations about ERK activation and Otx expression in the different cells wild type and mutant embryos. Sensitivity analyses and computations of Hill coefficients allowed us to assess the robustness of the specification mechanism controlled by cell surface area and to identify the respective role played by each signaling input.

Tuesday 14th

09:05 – 09:45

Precise and scalable self-organization in mammalian pseudo-embryos

Thomas Gregor

Princeton University & Institut Pasteur

Gene expression is an intrinsically noisy process. However, the body plans across individuals of a given species result in precise and reproducible spatial patterns. The transformation of variability in transcriptional activity into reproducible and precise gene expression patterns has been demonstrated in animal models ranging from worms to vertebrates. In flies, for example, the precision of the macroscopic features of the body plan has been traced back to the fundamental limits of molecular noise set by physical principles. The spatial accuracy of these features is proportional to system size and sufficient to distinguish individual cells from their neighbors. However, we know little about such accuracy in mammalian development. Mammals are different, and patterning and gene regulation are thought to be more variable. I will present an overview of my lab's previous contributions to the above findings and then introduce our recent use of a novel in vitro model called “gastruloids” to revisit these questions in mammalian development. We demonstrate an intrinsic reproducibility of the self-organizing anteroposterior body axis in gastruloids, both for growth dynamics and gene expression patterns. The system exerts tight control over expression levels and positions pattern boundaries with single-cell precision. Gastruloid growth scales with the initial number of seed cells, and gene expression patterns scale precisely with system size. Our results reveal developmental precision, reproducibility, and size-scaling for a mammalian system that, unlike a fly embryo, is not constrained by fixed boundary conditions (i.e., an eggshell). Gastruloids develop in an artificial context where boundaries are created through spontaneous self-organization. No selection pressure has acted on the system for it to operate at such high levels of precision. These spontaneously emerging quantitative properties could thus be fundamental features of multicellularity, reaching across half a billion years of evolutionary change.

09:45 – 10:10

Tissue-scale mechanics control stem cell fate and positioning during epithelial development

Clémentine Villeneuve, Somiealo Azote3, Elisabeth Lawson-Keister, Kai Kruse, Lisa Wirtz, Matthias Rübsam, Hisham Bazzi, Carien M. Niessen, M. Lisa Manning and Sara A. Wickström; Affiliation: Department of Cell and Tissue Dynamics, Max Planck Institute for Molecular Biomedicine Münster, Germany

Max Planck Institute for Molecular Biomedicine

The development and maintenance of a functional tissue and organs require synchronized regulation of cell state transitions and dynamic spatial positioning of specific cell states. During embryogenesis, the skin epidermis gradually transitions from a single stem cell (SC) layer to a multilayered stratified epithelium through coordinated differentiation and upward movement of cells. However, what mechanisms trigger initial fate commitment and how cell fate transition and positioning

are coordinated during development remain poorly understood. We show, using single cell transcriptomics, embryo live imaging, mechanical testing and 3D vertex modelling, that during early phases of stratification, multilayering occurs independently of cell fate specification, driven by high proliferative activity and a fluid-like tissue state. Subsequently, as the connective tissue matures and cell density increases, the tissue gradually solidifies and the stem cell compartment separates from the suprabasal differentiated compartment through high interfacial tension. This mechanical phase transition coincides with emergence of a committed stem cell population, characterized by altered cell state but stem-like adhesive and mechanical properties. In a second transition, the committed cells undergo a cytoskeletal and adhesion molecule switch, likely facilitating upward movement of the differentiated cell. Interestingly, the early fate commitment of basal stem cell is triggered by Notch signaling, reported to be modulated by cell geometry. Collectively, this work suggests two, mechanically distinct phases of epithelial barrier formation: (1) rapid growth-driven development of a first suprabasal layer and (2) mechanical separation of stem cell and suprabasal compartments requiring a Notch signaling-dependent early fate commitment to facilitate the transition of differentiated cells across this mechanical barrier to the upper layers.

10:10 – 10:50

Homeorhetic regulation of cellular phenotype

Aneta Koseska

MPI for Neurobiology of Behavior, Germany

11:30 – 11:55

A simple mathematical model to tease out links between tissue morphology and healing

Somya Mani, Tsvi Tlusty (Institute for Basic Science, South Korea); Affiliation: Konrad Lorenz Institute for Evolution and Cognition Research, Austria

Konrad Lorenz Institute for Evolution and Cognition Research

Tissues within a multicellular organism have diverse forms: while mammalian blood is fluid and disorganized, epithelial sheets are organized and contiguous. Concurrently, tissues look very similar across organisms: Consider animal epithelia versus the plant epidermis, or bundles of animal muscle versus plant vascular bundles. These similarities are striking because multicellular groups have evolved multiple times independently. Separately, a remarkable function common to many tissues is the ability to heal: even in organisms such as mammals, which are poor at regeneration, adult tissues routinely heal from injuries. The cellular organization within tissues, as well as the ability of tissues to heal result from developmental processes: cells divide, die, differentiate and migrate according to cues they receive from their neighborhoods. We ask two important and interlinked questions: How do multicellular organisms produce diverse forms of tissues using simple developmental processes? And how does tissue morphology relate to tissue regeneration? We address these questions using an agent based model of cell-fate where cells respond to different cellular neighborhoods using simple rules for cell division, death, differentiation and migration. Our model produces a rich diversity of tissue morphologies: by simply tuning the density of cellular interactions and the cellular propensity to differentiate, we can

produce tissues that are ordered and contiguous to those that are disorganized and disperse, and tissues that have sparse spatial arrangement to those that are densely packed. Intriguingly, tissue morphology was strongly predictive of tissue regeneration: the ability to heal was highly enriched in densely packed, contiguous tissues. Our work generates experimentally testable predictions on the effects of manipulating cellular interactions on tissue morphology and in turn, on tissue regeneration.

11:55 – 12:35

Signaling dynamics in the control of embryonic development and tissue homeostasis

Luca Giorgetti

FMI Basel, Switzerland

12:35 – 13:00

Manipulating Fate Decision Landscape in Single-Cell Aging

Lev Tsimring, Zhen Zhou, Yuting Liu, Yang Li, Yanfei Jiang, Nan Hao, Jeff Hasty, Lorraine Pillus; Affiliation: University of California, San Diego

University of California, San Diego

In our studies of single-cell aging dynamics of yeast S. cerevisiae, we found that isogenic cells diverge towards two aging paths, chromatin instability and mitochondrial decline, with distinct phenotypic changes and death forms. We identified two molecular pathways driving each aging fate and revealed that these pathways dynamically inhibit each other to form an early-life toggle switch that governs the aging fate decision. We show that the interactions between the chromatin silencing and mitochondrial pathways lead to an epigenetic landscape of yeast replicative aging with multiple equilibrium states that represent different types of terminal states of aging. Our quantitative model predicted that varying certain system parameters or rewiring the control circuit may result in profound changes of this landscape and emergence of novel equilibrium states or persistent oscillations that would affect the dynamics of cellular aging. Guided by these predictions we engineered two novel yeast strains indeed characterized by significantly extended replicative life span.

14:30 – 15:10

Shaping developing tissues via dynamic signalling grandients

Diana Pinheiro, Affiliation: IMP

IMP, Austria

Embryo development requires biochemical signalling to generate patterns of cell fates and active mechanical forces to drive tissue shape changes. However, how these processes are coordinated, and how tissue patterning is preserved despite the cellular flows occurring during morphogenesis, remains poorly understood. Gastrulation is a crucial embryonic stage that involves both patterning and internalization of the mesendoderm germ layer tissue. Here we show that, in zebrafish embryos, a gradient in Nodal signalling orchestrates pattern-preserving internalization movements by triggering a motility-driven unjamming transition. In addition to its role as a morphogen

determining embryo patterning, graded Nodal signalling mechanically subdivides the mesendoderm into a small fraction of highly protrusive leader cells, able to autonomously internalize via local unjamming, and less protrusive followers, which need to be pulled inwards by the leaders. The Nodal gradient further enforces a code of preferential adhesion coupling leaders to their immediate followers, resulting in a collective and ordered mode of internalization that preserves mesendoderm patterning. Integrating this dual mechanical role of Nodal signalling into minimal active particle simulations quantitatively predicts both physiological and experimentally perturbed internalization movements. This provides a quantitative framework for how a morphogen-encoded unjamming transition can bidirectionally couple tissue mechanics with patterning during complex three-dimensional morphogenesis

15:10 – 15:35

Modular, evolvable landscapes of cellular differentiation

Victoria Mochulska, Paul François (Université de Montréal and Mila Quebec); Affiliation: McGill University Quebec

McGill University Quebec

The epigenetic landscape of cellular differentiation, a long-known paradigm in developmental biology (Waddington, 1957), has evolved in the era of single-cell measurements into a quantitative, dynamical systems description. Cellular differentiation is modeled in a low-dimensional state space, with attractors corresponding to cell types, and transitions and bifurcations underlying cellular decisions. While it has become clear that such an approach can produce interpretable and predictive models (Corson and Siggia, 2012, Camacho-Aguilar et al, 2021, Sáez et al, 2022), current applications are designed in a largely ad hoc manner. We thus propose a method for systematic generation of landscape models for various systems. For constructing the landscape, we use local geometric features describing gradient and non-gradient dynamics. The building blocks of our model are at the scale of individual cell types, so the resulting landscape topology is flexible and subject to optimization along with the landscape parameters. This allows us to use minimal assumptions on the geometry of the landscape and the allowed differentiation routes. We verify that our model can generate the relevant topologies and standard bifurcations implicated in cellular decisions. We then use an evolutionary algorithm to optimize landscapes based on data. I will present the application of our method to two systems (neuromesoderm differentiation and the segmentation clock). For these examples, we can obtain landscapes different from pre-existing solutions (Sáez et al, 2022, Jutras-Dubé et al, 2020). Based on these results, I will discuss how experimental data and explicit or implicit assumptions on the studied system constrain the landscape model.

15:40 – 16:20

Intestinal cell fate dynamics in space and time

Sander Tans

AMOLF Institute, The Netherlands

Organoids are a major tool to study tissue renewal. However, characterizing the

underlying differentiation dynamics in space and time remains challenging. To address this need, we developed TypeTracker, which identifies cell fates by AI-enabled cell tracking and propagating measured endpoint fates back along the branched lineage trees. TypeTracker provides cell fates during growth and division for all cells, which allows us to pinpoint the moments and locations where cells commit to a new fate for all major cell types. Application to intestinal organoids indicates a new 'commit-then-sort' model of cellular differentiation, which contrast with the conventional conveyor belt picture where cells differentiate when moving up the crypt-villus axis. Specifically, cells that ultimately migrate to the villus in intestinal organoids commit to their new type early, when still deep inside the crypt, which has a number of important consequences: 1) Secretory cells commit before terminal division, with secretory fates emerging symmetrically in sister cells. 2) Different secretory types descend from distinct stem cell lineages rather than an omni-potent secretory progenitor. 3) The ratio between secretory and absorptive cells is strongly affected by proliferation of absorptive cells after commitment. 4) Spatial patterning occurs after commitment through type-dependent cell rearrangements. Our approach and resulting model provide a new perspective on intestinal cell fate dynamics, raises new questions about the underlying commitment and sorting mechanisms, and may be used more broadly to study spatio-temporal differentiation programs in diverse organoid systems.

16:20 – 16:45

Bonsai: Tree-based analysis of single-cell transcriptomics and epigenomics

Daan De Groot, Sarah Morillo Leonardo, Erik van Nimwegen; Affiliation: Biozentrum, University of Basel

Biozentrum, University of Basel

The study of cell fate decisions would be greatly aided by the accurate measurement of gene expression in single cells during differentiation processes. Single-cell transcriptomic (scRNA-seq) and epigenomic (scATAC-seq) methods promise to provide such measurements, but their analysis is complicated by the data's sparsity, high-dimensionality, and heterogeneous noise properties. Common analyses consist of mixtures of ad hoc methods with parameters that can be tweaked until results match prior expectations. Currently, there is not even a method for creating an unbiased visualization that facilitates data exploration, such that most publications use uninterpretable embeddings (e.g. UMAP) that are known to distort existing structures and to hallucinate non-existing ones. We propose that these challenges can be largely overcome by representing single-cell data in tree structures, such that distances measured along the tree meaningfully represent the similarities between cells. First, relations between objects in high-dimensional spaces can generically be captured well in tree structures. Second, for many biological datasets, we know that the data derive from an underlying cell lineage tree. Finally, tree structures can always be faithfully visualized in two dimensions. We therefore present Bonsai, a Bayesian inference method that, based on scRNA-seq data, reconstructs the tree that best captures the similarities between cells. Unlike other single-cell analysis methods, Bonsai carefully models the noise properties of the data and derives the optimal tree without any tunable parameters. The resulting trees are unbiased by prior expectations and can be visualized and explored in an interactive app. We show that Bonsai vastly outperforms common visualization tools on simulated data and can lead to new insights on real datasets. Moreover, the general and explicit formulation of the model

facilitates its extension to other datatypes such as bulk-RNA-seq and scATAC-seq.

Wednesday 15th

09:05 – 09:45

Signaling dynamics in the control of embryonic development and tissue homeostasis

Katharina Sonnen

Hubrecht Institute, The Netherlands

Cells communicate with each other via dynamic signalling pathways to govern development and tissue homeostasis. For instance, segmentation of vertebrate embryos is coordinated by oscillating signaling pathways. To enable the investigation of such dynamics, we established a microfluidic system to entrain endogenous signaling oscillations to external pulses of pathway modulator. Combined with real-time imaging of signaling reporters this enables the functional dissection of complex dynamic signaling networks in a multicellular context. Here, I will present our findings on the control of mouse segmentation by multiple interacting oscillators and how we have adapted such methods to study signalling dynamics in the context of the small intestine.

09:45 – 10:10

Unexpected differentiation patterns during local regeneration of hair cells in the inner ear

David Sprinzak, Shahar Kasirer, Olga Loza; Affiliation: George S. Wise, Faculty of Life Science, Tel Aviv University

Tel Aviv University

The mammalian balance system contains five vestibular sensory domains with alternating patterns of sensory hair cells (HCs) and non-sensory supporting cells (SCs). These alternating patterns are considered to emerge through the process of Notch mediated lateral inhibition. Many non-mammalian species have the capability to regenerate lost HCs by the proliferation and differentiation of nearby SCs. In contrast, in mammals the capacity to regenerate HCs quickly diminishes with age. However, little is known about the factors controlling regenerative processes in the vestibule and how lateral inhibition is employed to induce trans-differentiation of from SCs to HCs following HC death. Using a live imaging assay for mouse vestibular explants we track normal development and regeneration processes over extended periods. We show that vestibular sensory domain development is a highly dynamic process, where SCs exhibit cell divisions, delaminations, and differentiation events. We find that SCs to HCs differentiation events during early development (E17.5) typically obey the rules of lateral inhibition, namely, that newly differentiating cells do not have any HC neighbors. In contrast, at later stages (P0) differentiating cells typically have one HC neighbor, a behavior that does not align with standard lateral inhibition models. Moreover, a similar behavior is observed during local regeneration experiments where trans-differentiation of a SC into HC following laser ablation of single HCs at later stages does not match lateral inhibition. Finally, we show that

treating the explants with Rho Kinase inhibitor dramatically affects the dynamics of HC differentiation and trans-differentiation, suggesting a coordination between mechanics and differentiation. Our findings suggest that local HC regeneration cannot be explained by the classic lateral inhibition model and that local factors, including cell mechanics, play an important role during the process.

10:10 – 10:50

Rigidity-driven tissue epithelialization as a regulator of length-scales and time-scales of morphogen gradients

Nicoletta Petridou

EMBL Heidelberg, Germany

Abrupt transitions between solid-like and fluid-like tissue material states are essential for tissue shaping. However, if material phase transitions are instructing cell function is still debatable. Here, we show that tissue rigidification directly impacts signal transduction by regulating the length-scales and time-scales of extracellular molecular transport. By combining rigidity percolation theory, quantitative imaging analysis, genetics and optogenetics during zebrafish germ layer formation we uncover that a tissue rigidity phase transition impacts the spatiotemporal dynamics of meso-endodermal specification by sealing the paths of extracellular Nodal morphogen transport. This is a self-generated mechanism of morphogen gradient formation where Nodal itself, besides triggering meso-endodermal specification, increases cell-cell adhesion strength via regulating the expression of planar cell polarity genes. Once adhesion strength reaches a critical point it triggers a rigidity percolation transition which in turn minimises tissue porosity and induces the formation of tricellular contacts, resembling a transient tissue epithelialization. This rigidity-driven tissue epithelialization feeds back to morphogen signalling both at its length-scales, by restricting its extracellular transport, and at its time-scales, by facilitating its capture and degradation, resulting in robust pattern formation. Overall, we reveal how tissue phase transitions set the spatiotemporal dynamics of morphogen gradient formation and uncover macroscopic mechanisms of positional information.

11:30 – 11:55

Quantitative guiding of developmental cell-fates transitions using gene-free modelling

Wolfgang Keil, Ismail Hajji, Eric D. Siggia, Francis Corson; Affiliation: Institut Curie, Paris

Institut Curie, Paris

During development, cells gradually assume specialized fates via changes of transcriptional dynamics in thousands of genes. Terminal cell identities are then stabilized through the convergence of gene regulatory network dynamics and the accumulation of epigenetic DNA modifications. “Gene-free” (or geometric) modeling approaches for cell-fate acquisitions which abstract from the underlying gene regulatory landscape and reason in phenotypic space have been remarkably successful in explaining terminal fate outcomes. However, their implications for cellular dynamics during fate acquisition processes have so far not been tested in-vivo. To do so, here we combine gene-free mathematical modeling of cell-fate

acquisition during C. elegans vulval development with temporally controlled perturbations of in-vivo signaling dynamics using temperature sensitive (ts) mutant alleles of the EGF/Ras/MAPK and Notch signaling pathways. We show that gene-free modeling can quantitatively predict non-intuitive fate outcomes in variety of ts-genetic backgrounds, including pathway epistasis effects. In addition, we use gene-free modeling to infer how cell-fate transitions can be guided towards specific fate outcomes through timed pulses of signaling activity and verify these model predictions quantitatively with temporally controlled signaling perturbations via temperature shifts in ts backgrounds. Our results highlight the predictive power of gene-free models beyond terminal fate outcomes and illustrate a new approach to quantitatively guide cell-fate acquisition in a developmental context.

11:55 – 12:35

Stem Cell Institute, University of Cambridge
Maria Alcolea

12:35 – 13:00

Extracellular matrix mechanics regulates BMP signalling through a switch in epithelial organisation in human pluripotent stem cells

Ana Raffaelli, Tom Wyatt, Ewa Paluch, Kevin Chalut; Affiliation: Cambridge Stem Cell Institute, Department of Physiology, Development and Neuroscience, University of Cambridge

University of Cambridge

Historically, most research on cell fate induction was focused around biochemical signals, however, it is now clear that mechanical signalling from the extracellular matrix (ECM) also influences cell fate. For example, in human pluripotent stem cells (hPSCs), softening of the substrate increases mesoderm differentiation. We investigate the mechanisms underlying this phenomenon. To investigate the role of mechanical environment on hPSC fate, we exposed these cells to various stiffnesses using polyacrylamide hydrogels and subjected the cells to mesoderm-inducing BMP4 signal. We found that on soft substrates, hPSCs exhibit higher levels of BMP signalling activity. We showed that increased BMP signalling starts with lower Focal Adhesion Kinase (FAK) activation, and consequently lower activation of PI3K. Lower FAK-PI3K activity on soft substrates causes a less evenly-organised epithelium, in comparison to a more hexagonal cellular arrangement on stiff substrates. We hypothesised that these morphological changes represent alterations of epithelial functional properties. Indeed, a tight junction (TJ) protein Claudin-6 displays reduced junctional localisation in response to lower FAK-PI3K activity on soft substrates. Concomitantly, we also observed lower activity of apical actomyosin, whose force regulates TJ barrier. Finally, we asked whether these differences in TJ organisation are indicative of differences in epithelial permeability. To directly assess this, we exposed live cells to fluorescent dextran. We found that on soft substrates, hPSC epithelia exhibit higher permeability to BMP4-sized dextran. We are now investigating whether alongside permeability, epithelial polarity is also affected in response to the substrate. Together, our work identifies a mechanism through which mechanical signalling from the substrate intertwines with biochemical signalling to affect cell fate in hPSCs. We are also aiming to investigate whether such mechanism is involved in initiation of gastrulation during basement membrane remodelling. In general, our findings impact our understanding

of the role of ECM properties on biochemical signalling in fate transitions.

09:05 – 09:45

Transcriptional hubs as an agent for robustness and evolvability

Justin Crocker

Enhancers drive complex spatiotemporal patterns of gene expression during development, and their evolution is an important driver of morphological and phenotypic diversification. Recently, we found that random point mutations in the E3N enhancer of shavenbaby resulted in changes in the level, timing, or location of its activity, suggesting that the enhancer is densely encoded with regulatory information. To explore how such dense encoding of regulatory information might constrain the evolution of this enhancer, we systematically explored all the possible evolutionary paths between the modern-day E3N enhancers of D. melanogaster and simulans. Consistent with our previous results, we found extensive higher-order epistatic interactions across these evolutionary trajectories, with many paths reducing transcriptional outputs. Strikingly, interchromosomal interactions between different enhancer alleles resulted in synergistic or antagonistic effects on gene expression, in some cases driving novel expression patterns. Furthermore, we found evidence that these interactions are reinforced through transcriptional hubs. Therefore, beyond the previously established role of transcriptional hubs promoting developmental robustness, we propose that interallelic interactions between enhancers enable the exploration of novel phenotypes. Consequently, interchromosomal interactions may provide an additional layer for natural selection to act on regulatory evolution.

09:45 – 10:10

Theory of collective cell fate decisions in intestinal organoids

David Brückner, Cornelia Schwayer, Silvia Barbiero, Edouard Hannezo, Prisca Liberali; Affiliation: Institute of Science and Technology Austria

Institute of Science and Technology Austria

To form a functionally complex organ, cells sense external signals and integrate them with their intrinsic properties to determine their cell fate. A prime example of cell-cell interactions driving organ patterning are intestinal organoids, in which a population of uncommitted progenitors undergoes a symmetry breaking event to result in stem cell niche formation and crypt-villus axis emergence. Previous work showed that this symmetry breaking is driven by Dll1/Notch lateral inhibition, but also requires heterogeneous activity of Yap1. However, a conceptual framework to understand how cell-cell communication and cell-intrinsic heterogeneity result in complex fate choices is lacking. To bridge this gap, we develop a biophysical model of collective cellular decisions, combining lateral inhibition, heterogeneous cellular states, and active epithelial mechanics. Using a set of perturbation experiments including Yap1 inhibition and overexpression to constrain the model, we make key quantitative predictions for cellular states during symmetry-breaking, including a prepatterned Dll1 state based on Yap1 activity. By combining high-throughput imaging and single-cell omics, we confirm our predictions and reveal how cells integrate the cell- and tissue-scale signals into the prepatterned Dll1 state via FoxA transcription factors, through extensive epigenetic remodeling of secretory progenitor cells. Taken together, we demonstrate how minimal biophysical models can make key predictions for stem cell fate choices in

complex multicellular systems. This approach reveals how cells sense multimodal signals including biochemical and metabolic signals and integrate this information with tissue-level mechanical properties to take the first cell fate decision, break symmetry and properly pattern intestinal organoids.

10:10 – 10:50
Zena Hadjivasiliou

Francis Crick Institute, UK

11:30 – 11:55
Dynamics of morphogen source formation in growing tissues
Marcin Zagorski, Richard Ho, Kasumi Kishi, Maciej Majka, Anna Kicheva; Affiliation: Institute of Theoretical Physics, Jagiellonian University

Institute of Theoretical Physics, Jagiellonian University

Understanding the biophysical mechanisms governing morphogen gradient source formation is crucial for reproducible and organized organ development. Although many genetic interactions involved in the establishment of morphogen production domains are known, the biophysical mechanisms of morphogen source formation are poorly understood. In this talk I will address this by focusing on the morphogen Shh in the developing spinal cord. Shh is produced by the adjacently located notochord and by the floor plate of the spinal cord. Through a data-constrained computational screen, we identify distinct mechanisms of floor plate formation, with only one consistent with experimental data. Our study reveals that the floor plate establishment involves a rapid response to Shh from the notochord and regulatory interactions within the spinal cord. Key regulatory elements such as uniform activators and Shh-dependent repressors drive the establishment of the floor plate size. Following the initial establishment, the floor plate becomes insensitive to Shh and expands in response to tissue growth, leading to proportional scaling with spinal cord size and Shh amplitude. This separation of time scales ensures robust and growth-dependent floor plate formation. Our findings suggest a common strategy for scaling morphogen gradient amplitudes in growing organs, with implications for understanding developmental pattern scaling. Moreover, our model provides a quantitative framework for studying morphogen source formation dynamics in diverse growing tissues.

11:55 – 12:35
Spatial and temporal order in the developing Drosophila eye
Francis Corson

ENS Paris, France

There are many instances in development where a regular arrangement of cell fates self-organizes through cell-cell interactions, yet the dynamics by which these patterns arise, and the underlying logic, often remain elusive. In the developing Drosophila eye, regular rows of light-receiving units emerge in the wake of a traveling differentiation front to form a crystal-like array. The propagation of this pattern is thought to proceed by templating, with inhibitory signaling from each row providing a negative template for the next, but its dynamics had not been directly observed. Live imaging reveals

unanticipated oscillations of the proneural factor Atonal, associated with pulses of Notch signaling activity. Our observations inform a new relay model for eye patterning, in which dynamic signaling from row n triggers differentiation at row n+2, conveying both spatial and temporal information to propagate crystal-like order.

12:35 – 13:00

Enhancer cooperativity can compensate for loss of activity over large genomic distances

Christa Bücker, Henry Thomas, Songjie Feng, Felix Haslhofer; Affiliation: Max Perutz Labs, University Vienna

Max Perutz Labs, University Vienna

Enhancers are short DNA sequences that regulate target gene from a distance and in spatio-temporal patterns. The expression of many genes is controlled by combinations of multiple enhancers, but the interaction and cooperation of individual enhancer elements is not well understood. Here, we developed a novel synthetic platform in mouse stem cells that allows building complex regulatory landscape from the bottom up. We tested the system by integrating individual enhancers, which are active in early development stage, at different distances and confirmed that with increasing distance to the promoter, expression of the reporter gene decreased. However, the reduction level depends on the enhancer's intrinsic strength. Furthermore, introducing a weak enhancer between a strong enhancer and the promoter can partially rescue the decreased reporter gene expression. Therefore, synergy between enhancer elements can increase the genomic distance from which enhancers can function.

Selected poster presentations
Poster Session 1

Poster 1

Decoding Pattern Formation Mechanisms: The physics behind morphogen transport

Aguirre Tamaral Adrian; Affiliation: Statistical physics of living systems Group, University of Graz

University of Graz

During embryonic development, groups of cells are organized creating patterns to give rise to tissues and organs. Experimental discoveries have unveiled a plethora of mechanisms underlying cell fate and pattern formation, ranging from molecular pathways to different molecular transport processes. Pattern formation has been extensively investigated using simple reaction-diffusion (R-D) equations (Turing model) and various models that go beyond diffusion-based mechanism, incorporating experimental transport processes like filopodia-mediated signaling, molecular motor-driven transport and vesicle-mediated transmission, among others. The vast number of proposed mechanisms and models may seem overwhelming, suggesting that pattern formation is highly specific to each animal model, tissue type, transport mechanism, or morphogen molecule. However, pattern formation may not be confined to specific pathways or transport mechanisms and may be a generic framework for

cellular signaling and fate acquisition. For example, cytonemes (filopodial cellular channels) are prevalent across the main signaling pathways, delivering signaling molecules and vesicles via filopodia-like structures, regardless of the biochemical differences between morphogens. Supporting this idea, we created a biophysical model to study cytoneme signaling and guidance during development (Aguirre-Tamaral et al. Nat. Commun 2022) and we showed that cytoneme signaling and diffusion-based mechanisms can produce similar signaling patterns (Aguirre-Tamaral et al. PLoS Comput. Biol 2021) and may be formally described with similar R-D equations, implying that evolution focuses on the emergent pattern rather than the specific formation mechanism. We propose that there exists a unifying framework for pattern formation, which may encompass the mechanisms observed across different biological systems. Studying this new framework with more general R-D equations would not replace the specific mechanisms themselves, but would rather provide a higher-order understanding of how pattern formation emerges. References: - Aguirre-Tamaral et al. Predictive model for cytoneme guidance in Hedgehog signaling based on Ihog- Glypicans interaction". Nature Communications (2022) - Aguirre-Tamaral et al. "Improving the understanding of cytoneme-mediated morphogen gradients by in silico modeling." PLOS Computational Biology (2021)

Poster 2

Cell patterning in the the intestinal crypt

Betjes Max, Lidewei Dubbink, Jeroen van Zon, Sander Tans; Affiliation: AMOLF

AMOLF

The stem cell zone of the intestinal crypt consists of two cell types; stem cells and Paneth cells. These Paneth cell are the major source of Wnt-protein and are therefore essential for niche maintenance. In the crypt Paneth cells are interspersed between stem cells in a 'checkerboard'-like pattern to optimize signaling. This pattern is thought to be established by Paneth cells inhibiting Paneth differentiation in their neighbors (lateral inhibition) through Notch signaling. The Notch pathway is responsible for similar patterns throughout animal development from the drosophila wing to the mouse auditory epithelium. In contrast, we find that pattern formation through lateral inhibition is impossible in the crypt. During long term live imaging, we see that the constant divisions of the stem cells constantly disturb the patterning. This makes the intestine fundamentally different from 'developmental' systems where the pattern only has to be established once. Instead we hypothesize that Paneth cells actively separate from each other when they become neighbors. We validate this thesis by disturbing Paneth cell patterning by targeted photo-ablation of single cells in the crypt, and following the pattern reestablishment. From these experiments we also show that Paneth cells are stiffer then stem cells. Furthermore, through laser-cutting of cell interfaces, we show that membrane tension between cells is dependent on cell type. We conclude that these differential mechanical properties, and not lateral inhibition through signaling, likely give rise to the 'checkerboard'-like pattern. In conclusion, our study establishes a new mechanical way of pattern formation that can overcome the specific challenges posed by the constantly self-renewing epithelium of the intestine.

Poster 3

Single plant cells: from destruction to reconstruction

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The plant cell wall is essential for plant growth, cell division, morphogenesis, cell-cell adhesion, and fate. To better understand the role of the plant cell wall in these processes outside of a complex tissue context, we aim to study isolated cells. We can extract protoplasts (plant cells without a cell wall) by digesting the cell wall with enzymes. However, protoplasts are not “real plant cells” as they need the cell wall to develop. Although it is quite straightforward to obtain protoplasts, it is significantly more delicate to monitor efficient cell wall regeneration. To form the cell wall, the plant cell secretes polysaccharides around itself that come together to form a continuous layer. In liquid culture, the polysaccharides are excreted outside of the cell but are not efficiently retained around it. This could explain the low efficiency of cell wall regeneration. Here, we are using two different approaches to increase cell wall regeneration efficiency. First, I am optimizing the cell wall regeneration medium and the culture conditions. The cell wall regeneration protocol is very lab dependent, therefore I am developing a quantitative approach to screen numerous conditions quickly. Second, I am coating the protoplasts with a layer-by-layer method. The coating provides a first mesh to retain the secreted polysaccharides, and therefore, we expect an increase in the regeneration efficiency. An efficient cell wall regeneration protocol to obtain single plant cells is the first step to study the plant cell fate in vitro. Without a cell wall, plant cells de-differentiate. After cell wall regeneration we can modify their chemical and/or physical environment to induce the differentiation into different cell types, which makes them a perfect model to study cell fate in vitro.

Poster 4

Optimal regimes of regulatory sequence evolution

Borbely Reka, Michal Hledík, Gašper Tkačik

ISTA

Cis-regulatory elements, such as enhancers and promoters, control gene expression by binding regulatory proteins such as transcription factors. In contrast to their bacterial counterparts, metazoan CREs typically bind TFs with short recognition motifs across multiple functional yet often weak binding sites. The evolutionary origin of this architecture remains unclear. Here we use simulations and information-theoretic arguments to study adaptive evolution of entire CREs under selection for regulatory phenotypes. In a biophysical toy model that recapitulates the essential nonlinearities of metazoan regulation, a regulatory phenotype requires a gene to be active when its CRE binds cognate TFs, yet inactive in the presence of noncognate TFs that can cause regulatory crosstalk. We explore evolutionary outcomes for CREs assuming an "adapt-and-optimize" approach, where CRE evolution is tracked explicitly at the sequence level on a fast timescale, while the biophysical model itself -- the genotype-phenotype map -- is numerically optimized for evolvability of CREs. In the optimal regime, selection navigates the tradeoff between slowly evolving strong and long binding sites (which guarantee low crosstalk), and rapidly evolving multiple short and

weak binding sites (which necessitate diffuse selection against noncognate binding across the CRE): here, functional CREs emerge rapidly and naturally contain a diversity of strong and weak binding sites, as reported in empirical studies. We argue that in strongly interacting systems, where studying the evolution of an isolated component could be misleading but studying the co-evolution of all components intractable, the "adapt-and-optimize" provides a powerful approach to understand the evolution of biological complexity.

Poster 5

Robustness of pattern proportions during growth

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Francis Crick Institute

Morphogens are molecules that form gradients across developing tissues, and guide growth and patterning. As growth and patterning occur concomitantly, the domain sizes of patterned regions and the tissue size are dynamic quantities that co-vary. Despite noise affecting these processes and a natural variability in size between individuals, the variation in patterning proportions between embryos is significantly lower than the constituent variations in tissue or domain sizes. One method to achieve this robustness in pattern proportions is a scaling mechanism, where the lengthscales of pattern and growth are maintained to be proportional despite both evolving in time. Active scaling mechanisms have been observed at the level of the morphogen gradient, however, this is not observed in all developmental contexts. Here we ask, in what cases is having a scaling mechanism important? We have set up a system without an active scaling mechanism and investigated how much robustness is exhibited in the patterning proportions over developmental time. We have performed a phase space analysis for the parameters controlling the timescales of patterning and growth, and found there is a limited and finetuned section of parameter space where the patterning proportions can be robustly defined despite variation in time of pattern specification. We have integrated experimental data to understand where different model systems lie in this parameter space, and whether we can rationalise the presence or absence of scaling mechanisms. We are expanding this analysis to include Gene Regulatory Networks, to explore if the dynamics of GRNs may be a critical component in the emergent robustness in defining pattern proportions.

Poster 6

Studying the underlying principles of organ size control using restorative growth assay in vitro

Böhm Sebastian

ISTA

In developmental biology, a key question is how animals develop organs of the correct size. This process requires precise coordination between organ growth and morphogenesis to ensure a properly formed body plan, as deviations can severely affect survival. Additionally, organ growth must be adaptable to cope with

environmental changes like injuries or fluctuating food supplies. Historically, the regulation of embryonic growth has been linked to nutritional factors, with studies showing mammals can undergo accelerated growth to catch up after periods of nutritional deficiency. Further research introduced the use of Mitomycin C, a teratogen that induces malformations, to explore growth recovery in E7 mouse embryos. This revealed that embryos can regenerate even after significant cell loss, indicating a critical period for size regulation during gastrulation. Our lab has developed a method to create 2D spinal cord organoids from mouse embryonic stem cells, allowing for detailed growth analysis under controlled conditions. This technique, which initiates growth on patterned surfaces before allowing expansion, is particularly suited for studying how organoids recover from disturbances like Mitomycin C exposure. Preliminary data suggest that these organoids can regenerate to their original size within 72 hours after treatment. We aim to further investigate the cellular mechanisms behind this restorative growth through techniques like immunostaining and live imaging to understand cell proliferation and death rates. By examining how embryonic tissues compensate for growth disruptions, we hope to gain insights into the fundamental principles of organ size regulation and regeneration.

Poster 7

Temporal Dynamics of Morphogen-Encoded Positional Information at Single-Cell Resolution During Tissue Morphogenesis

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Washington University in St. Louis

Positional information conveyed by morphogen gradients is essential for directing cell fates during tissue patterning. In the developing zebrafish spinal cord, opposing gradients of Sonic hedgehog (Shh) and Bone morphogenetic protein (BMP) specify 13 distinct cell types along the dorsal-ventral axis. This cell fate specification occurs within a dynamic structure, as the spinal cord evolves from a flat, two-dimensional plate to a three-dimensional cylinder, accompanied by extensive cellular movements and neighbor exchanges. Through in toto imaging, we mapped the trajectories of every cell within a 300 μm segment of the zebrafish spinal cord from the neural plate stage (0-somite) to the neural tube stage (12-somite). Our bespoke deep-learning model, optimized for high-precision nucleus segmentation, enabled us to reconstruct the positional histories and quantify Shh signaling responses for the entire ventral spinal cord cell population (p3-p0), comprising over 350 cells from more than 200 lineages per embryo at single-cell resolution. Unexpectedly, we discovered that positional information, as encoded by Shh signaling responses, did not progressively increase. Instead, it peaked at approximately 1.5 bits at the 4-somite stage and subsequently declined to 1.1 bits by the 6-somite stage, coinciding with neural keel formation. This plateau persisted without notable recovery until the 12-somite stage, when cellular movement had decreased following neural tube formation. The decline in positional information is primarily attributed to cellular rearrangements and a heterogeneous response to the Shh gradient, which complicates the restoration of positional precision. Remarkably, inhibiting Shh signaling at later stages of spinal cord patterning, when endogenous Shh signaling is noisiest, significantly enhanced overall positional information. Our study provides insights into the complex dynamics of

morphogen-encoded positional information and establishes a foundation for future research on patterning mechanisms during tissue morphogenesis.

Poster 8

Unveiling Gene Perturbation Effects through Gene Regulatory Networks Inference from single-cell transcriptomic data

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Università di Padova

Understanding how the genes interact and organise themselves into Gene Regulatory Networks (GRN) to influence complex cell dynamics is challenging. Experimental gene perturbations allow for inspecting GRN changes and the resulting cell phenotype. However, they are limited by the sheer number of possible gene targets. In silico models offer broader, time-efficient alternatives to reveal key regulatory mechanisms. Current methods can infer GRN interactions, generate unperturbed data or study how perturbations propagate in the GRN and modify cell phenotypes. However, these models do not focus on the link between GRN functioning and cell phenotypes. We propose an unsupervised machine learning approach, IGNITE, Inference of Gene Networks using Inverse kinetic Theory and Experiments. It assumes as GRN model the kinetic Ising model from statistical mechanics. IGNITE can (1) start only from unperturbed single-cell RNA sequencing (scRNA-seq) data without needing additional input data (e.g. ATAC-seq), (2) infer directed, weighted, and signed GRN that best describes observed data, (3) generate unperturbed patterns of gene (de)activation that describe specific cell phenotypes, and (4) simulate perturbations, such as multiple genes knockouts, forecasting the resulting perturbed cell phenotype. We applied IGNITE to murine Pluripotent Stem Cells progressing from naive to formative state during early development. It simulated the knockout of three genes (Rbpj, Etv5, and Tcf7l1), individually and simultaneously, forecasting the gene activity changes for the remaining genes in the GRN compared to the unperturbed states. Strikingly, these simulations were largely supported by experimental data and outperformed the ones obtained with SCODE and CellOracle, the two current gold standard methods. IGNITE captures the functional complex organisation inside the GRN linked to specific cell behaviours. Overall, the findings suggest that our model is a promising tool for studying gene perturbations and could lead to new insights in describing the effects of GRN interaction on cell phenotypes.

Poster 9

Is the cellular jamming transition universal?

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University of Vienna

Phase transitions and collective motion play a crucial role in various biological processes, such as embryogenesis, wound healing, and cancer progression, during which tissues can transition between states resembling liquids or solids. A well-known case is that of a sub-confluent epithelial monolayer that approaches homeostatic equilibrium by undergoing a liquid-to-solid transition, sometimes referred to as

jamming. Seminal work by Angelini et al. (doi:10.1073/pnas.1010059108) suggested that this dynamical arrest of the monolayer resembles a glass transition, primarily driven by the increasing cell density due to proliferation. Garcia et al. (doi:10.1073/pnas.1510973112) found that cell density plays a secondary role, suggesting that the dynamical arrest can be rationalized in terms of the combined maturation and strengthening of cell−cell and cell−substrate adhesions. To shed light on these contrasting results, we combine Particle Image Velocimetry, Segmentation Analysis, and Differential Dynamic Microscopy to perform a multi-scale investigation of the monolayer dynamics across the jamming transition. Our preliminary results reveal a lack of uniformity in how different cell lines undergo jamming, showing dramatically different behaviors depending on the cell type. While at early stages all cell types exhibit a ballistic-like, directional motility phenotype, some of them jam by just slowing down, whereas some others exhibit an additional transition from a ballistic to a diffusive type of motion. In addition, we find different degrees of interplay between the monolayer dynamics and its structural features. The observed differences in behavior are not only evident between distinct cell types (e.g., epithelial cells versus fibroblasts) but also among different lines of the same type, suggesting that the cellular jamming transition is not universal.

Poster 10

Mechanochemical feedback drives neuron-like signaling dynamics

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European Molecular Biology Laboratory Heidelberg

Temporal dynamics of cell signaling are crucial for cell fate decisions. Cells can adjust their mechanics and adapt their shape in response to environmental signals, yet the impact of shape changes on signal processing and the associated feedback dynamics remain largely unexplored. Motivated by the mechanochemical feedback observed in multicellular systems, we describe cells as incompressible droplets adjusting their interfacial tensions in response to contact-dependent signals. We derived a minimal set of equations governing the mesoscopic droplet states controlled by just two dimensionless feedback parameters. The shape and signaling dynamics of adaptive droplets exhibit non-linear dynamics akin to those found in conductance-based models of neuronal excitation, including bistability, integrator versus resonator responses, various classes of excitability, and tunable shape oscillations ranging from near-sinusoidal to relaxation-type. Our tractable framework offers a new paradigm for understanding how soft active materials respond to shape-dependent signals and suggests novel modes for the generation of dynamic cell signals.

Poster 11

Precise and scalable self-organization in mammalian pseudo-embryos

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Institut Pasteur, Paris, France

Gene expression is inherently noisy, yet in various animal models, this noise is transformed into precise expression patterns. While such accuracy remains poorly

understood in mammals, we investigate this phenomenon using gastruloids, an in vitro model for early mammalian development. Our study aims to elucidate the regulation of self-organization in this system. We demonstrate that gastruloids exhibit remarkable intrinsic reproducibility in growth dynamics and gene expression patterns. Along the main body axis, expression levels are tightly controlled, and pattern boundaries are positioned with single-cell precision. Notably, as gastruloid size scales, body proportions are established with remarkable precision.

Poster 12

Pattern formation in tissues with heterogeneous motility and rigidity

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Institute of Theoretical Physics, Jagiellonian University

Understanding the formation of cellular patterns in developing tissues remains a fundamental question in developmental biology. Cellular motility, tissue mechanical properties, and tissue heterogeneity are closely related to cellular rearrangements. Here, we focus on studying how patterns defined by the group of cells with different mechanical properties than the properties of the surrounding tissue, change over time. We address this question by using the vertex model. This model represents tissue structure as a tessellation of polygons, with key parameters corresponding to cell motility, cell preferred area, and target shape index (normalized cell perimeter). By modifying the target shape index and motility we could study tissues with solid- or fluid-like properties. By considering a group of cells with distinct cell motility and target shape index embedded in the surrounding tissue we investigated their long-term fragmentation and shape. The results indicate less dispersion in more rigid groups of cells. The covariance of the cell centers was used as a descriptor of the elongation of the grouped cells. We find that the groups of rigid cells become more and more elongated between softer cells. The results indicate the critical role of mechanical parameters in tissue rearrangement and pattern formation in tissues containing groups of cells exhibiting different properties than the rest of the surrounding cells.

Poster 13

Regulation of spinal cord size during mouse development

Harish Rohit Krishnan

ISTA

A fascinating question in biology is how developing organs grow in size to achieve their correct dimensions in a stereotypical and reproducible manner. Previous experiments, in which the size of mammalian embryos was reduced in early development, showed that many organs, including the developing spinal cord, restore their normal sizes by mid-gestation. This implies that there are inherent mechanisms of tissue size determination that allow deviations in size to be sensed and restored. However, what these mechanisms are is unknown. In this project, we investigate the role of morphogen signaling gradients in tissue size control in the developing mouse neural tube. By designing spinal-cord specific perturbations in tissue size, we found that restorative growth occurs in a tissue-intrinsic manner. We further found that the loss of cells in the tissue is followed by a transient period of increased proliferation.

Preliminary data shows alterations in the anti-parallel Shh and BMP signaling gradients along the dorsoventral axis of the neural tube in embryos undergoing restorative growth.

Poster 14

Enhancer-enhancer interaction helps bridging genomic distance

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Max Perutz Labs

Multiple enhancers are typically involved in controlling the expression of developmental genes. These elements do not work as independent units but display a variety of interdependencies and hierarchies, exemplified by systematic dissections of enhancer clusters. Because each enhancer cluster is unique in composition, universal rules have yet to emerge. Current high-throughput approaches are either plasmid-based and lack the endogenous chromatin context or, when integrated, do not recapitulate dynamics caused by genomic distance between elements. To address this, our lab utilised a bottom-up approach to create a synthetic genomic locus in mESCs, consisting of a reporter gene (mCherry) and three landing pads at different distances (1.5 kb, 25 kb, and 75 kb). Using this system, we have observed a relationship between intrinsic enhancer strength and the ability to activate transcription from a distance. We could also show that strong and weak enhancers can work together to bridge genomic distance, resulting in a superadditive output. We are optimizing our integration system to integrate libraries of enhancers and hope to identify elements displaying varying modes of interaction. We will also elucidate how these elements “work together” mechanistically, including analyzing the 3D-genome, chromatin marks and transcription factor binding.

Poster 15

Cellular Compartmentalisation and Receptor Promiscuity as a strategy for Accurate and Robust Inference of Position during Morphogenesis

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Precise spatial patterning of cell fate during morphogenesis requires accurate inference of cellular position. In making such inferences from morphogen profiles, cells must contend with inherent stochasticity in morphogen production, transport, sensing and signalling. Motivated by the multitude of signalling mechanisms in various developmental contexts, we show how cells may utilise multiple tiers of processing (compartmentalisation) and parallel branches (multiple receptor types), together with feedback control, to bring about fidelity in morphogenetic decoding of their positions within a developing tissue. By simultaneously deploying specific and nonspecific receptors, cells achieve a more accurate and robust inference. We explore these ideas in the patterning of Drosophila melanogaster wing imaginal disc by Wingless morphogen signalling, where multiple endocytic pathways participate in decoding the morphogen gradient. The geometry of the inference landscape in the high dimensional

space of parameters provides a measure for robustness and delineates stiff and sloppy directions. This distributed information processing at the scale of the cell highlights how local cell autonomous control facilitates global tissue scale design.

Poster 16

Symmetry breaking and self-organization of bi-layered epithelia are orchestrated by conserved signals during development and regeneration

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Coordination of cell fate acquisition and morphogenesis is necessary to generate functional organs. During development, specialized cell types are generated from stem cells in a tightly regulated spatiotemporal manner to fulfil tissue functions. Yet, how stem cell differentiation and cell dynamics are orchestrated to generate tissues of complex shapes harboring defined cell types remains a major question in the field of development and cell biology. Several glandular epithelia such as prostate, mammary, salivary and lacrimal glands share common features. These tissues are branched bilayer epithelia that share an overall similar cellular hierarchy and start morphogenesis during embryonic development. Furthermore, these tissues are derived from stem cells that will give birth to progenitors at the onset of morphogenesis. We hypothesized that these similarities could be regulated by a common mechanism linking fate specification and branching morphogenesis. We developed a in vitro model recapitulating the different steps of the embryonic development of glandular epithelia in vivo. We used this experimental system with genetic or pharmacological perturbation and combined it with automatic quantification of fate markers staining or expression that allows us to precisely decipher the players involved in binary fate decision. We confirmed our results in ex vivo culture of embryonic tissues and demonstrated that 3D positional cues regulate the Yap and Notch pathways to achieve symmetry breaking of the tissues. We then investigated the conservation of our mechanism in other context of binary fate decision such as irradiation or ablation induced regeneration in the four tissues. Overall, our results indicate the existence of a strongly conserved axis of signaling linking the Yap, P63 and Notch involved in formation of bilayer epithelial tissues.

Poster 17

Regulation of size and shape of the notochord during mouse development

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The notochord is a rod-like organ that extends along the anterior-posterior body axis during embryonic development in vertebrates. This organ plays a central role in pattern formation by secreting signalling molecules that spread into the surrounding tissues. The dimensions of the notochord are relevant for its secretory function, yet how the size and shape of the notochord are controlled is poorly understood. To address this, we used deep tissue clearing to visualize and measure the notochord size and shape between E8.5 and E10.5 of mouse development. Our data revealed that the notochord

cross-sectional area is constant along the trunk of the embryo and increases in size in the posterior region of the embryo. The notochord maintains this funnel-like shape across developmental time as it increases its length. To understand how this is achieved, we studied the cell behaviours that affect notochord size and shape. We found that spatially non-uniform cell division rates in the notochord account for its increase in size over time. Live imaging of embryo explants further revealed that notochord cells undergo active cell migration, which leads to directional displacement of notochord cells relative to the neighbouring somites. Perturbation experiments suggest that cell migration is essential for maintaining the correct diameter and length of the notochord. Using biophysical modelling, we are currently investigating how cell proliferation, active cell migration and the deposition of notochord basement membrane interact to regulate notochord size and shape. These results will provide new insight into how the extension of the notochord is coordinated with other tissues while at the same time ensuring correct gradient formation of notochord-produced morphogens.

Poster 18

Nonself RNA rewires IFN-β signaling: A mathematical model of the innate immune response

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Type I interferons (IFNs) are key coordinators of the innate immune response to viral infection, which, through activation of the transcriptional regulators STAT1 and STAT2 (STAT1/2) in bystander cells, induce the expression of IFN-stimulated genes (ISGs). Here, we showed that in cells transfected with poly(I:C), an analog of viral RNA, the transcriptional activity of STAT1/2 was terminated because of depletion of the interferon-β (IFN-β) receptor, IFNAR. Activation of RNase L and PKR, products of two ISGs, not only hindered the replenishment of IFNAR but also suppressed negative regulators of IRF3 and NF-κB, consequently promoting IFNB transcription. We incorporated these findings into a mathematical model of innate immunity. By coupling signaling through the IRF3-NF-κB and STAT1/2 pathways with the activities of RNase L and PKR, the model explains how poly(I:C) switches the transcriptional program from being STAT1/2 induced to being IRF3 and NF-κB induced, which converts IFN-β-responding cells to IFN-β-secreting cells.

Poster 19

Deriving the phase diagram structure for optimal information integration in biological signaling systems

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Peking University, China

Information integration is widely presented in various biological processes. For example, the development of multicellular systems usually involved the readout and integration of multiple morphogen gradients. Response noise and response sensitivity

are two key features of information transmission channels, but their quantitative relationship remains to be characterized. While understanding the behavior of biological signaling systems usually requires ad hoc modelling with data-fitting, it has been proposed that the optimality principle could be applied in predictive modelling in a data-free manner. In our study, following the spirit of the normative theory [Młynarski et al., Neuron, 2021], we adopt a top-down, principle-based model in the language of "phase diagram" to study the steady state properties of information integration systems. We find that the response noise of integrating multiple signals, that are affected by static noise, is bound by the response sensitivity, consistent with the philosophy of the data-processing-inequality (DPI) in information theory. While DPI only states that such a limit exists, we derive the condition to achieve the optimal noise-sensitivity trade-off in information integration systems with static noise. Our results reveal that signaling networks with the optimal information integration can be better characterized by a phase diagram structure, rather than the widely-used network topology, offering an alternative method for predictive modelling. Furthermore, we identify the derived noise-sensitivity relation in the patterning gene expression data in early fly embryogenesis [Petkova et al., Cell, 2019] and find that the patterning network appears to be optimized. Based upon the optimal phase diagram model, we can quantitatively predict the patterning response to the dosage perturbation of morphogen Bicoid [Liu et al., PNAS, 2013] without parameter fitting. Our model provides a novel perspective in understanding information transmission in biological signaling systems.

Poster 20

New class of multimodal Turing patterns

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We present a new set of Turing patterns based on the superposition of multiple waves. Turing patterns are well known solutions to a set of reaction-diffusion equations. Such patterns have been studied in the context of embryo development, chemical reactions, nonlinear optics, ecology and random walks, to name a few. The main feature of systems giving rise to Turing patterns is that a stable state of reaction is destabilized when the reactants may diffuse. The system then generates a periodic solution with a dominant wavelength, which establishes the pattern. Additionally, the system provides examples of spontaneous symmetry breaking and self organization behaviour. Turing originally discovered his results when considering two morphogen species, the chemicals used by cells in an organism to communicate and grow into a pattern during development. The values of the kinetic and diffusion parameters leading to Turing patterns are known as Turing space. The degree and the number of polynomial inequalities defining Turing space, grow rapidly with the number of morphogens. Due to this, Turing patterns for more than two or three morphogens were mostly studied numerically on a case-by-case basis. Now, we developed a method to construct analytically a linear solution to the reaction-diffusion system for certain regions of Turing space. This linear solution corresponds to a system with a regulatory network of diffusible morphogens. By providing analytical insights into dynamics of the morphogens we are able to show the existence of new types of Turing patterns, and relate these patterns to the structure of the network. These patterns have two

dominant wavelengths in the case of four morphogenes. Moreover, we explain how it is possible to have superpositions of n wavelengths for 2n distinct morphogen species. Finally, we discuss possible implications of the obtained class of multimodal Turing patterns for establishing reproducible and precise patterns in biological systems.

Poster 21

AKT Regulates Pluripotency Transitions Through Gating of FoxO Transcription Factors

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Max Perutz Labs

Naïve pluripotency is sustained by a self-reinforcing gene regulatory network (GRN) comprising core and naïve pluripotency-specific transcription factors (TFs). Transitioning from this naïve state to a formative pluripotent state, reminiscent of early post-implantation development, represents a critical cellular fate decision that is crucial for generating an entire organism from a few pluripotent cells. The exit from naive pluripotency is instructed by a set of signalling pathways, but how changes in pathway-activities are translated into decommissioning the naïve and initiation of the formative GRN remains largely unknown. Our research identifies a critical and previously unexplored function of AKT signaling in controlling the nuclear localization of FoxO transcription factors to establish formative cell identity. Although FoxO-TFs are well-known regulators of longevity and metabolism, their involvement in cell-fate transition has been less examined. In naïve ESCs and in the naïve E4.5 epiblast in vivo, phosphorylated AKT prevents FoxO-TFs from entering the nucleus. At the onset of differentiation, PTEN-induced reduction of AKT-activity enables shuttling of FoxO-TFs into the nucleus, both in vitro and in the rosette-stage of the E4.75 epiblast. Inside the nucleus, FoxO-TFs enforce the transition from naïve to formative pluripotency by activating formative-specific enhancers and repressing naive-specific ones. This is consistent with a dual role for FoxO-TFs in decommissioning the naïve, while establishing the formative gene expression programme. FoxO1 binds to around 2,000 target enhancers and significantly overlaps with key pluripotency factors such as NANOG, OCT4 and OTX2 on chromatin. Remarkably, FoxO1 binding s a more reliable indicator of gene expression changes during differentiation than other pluripotency TFs, both in vitro and in vivo. Together, this highlights FoxO1 as novel pluripotency transcription factor with a crucial role in instructing early embryonic cell fate transitions. We further provide a mechanistic explanation for the role of AKT signalling during pluripotency state transitions.

Poster 22

Deciphering the self-organizing principles governing tumor growth and cellular composition

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IBDM

Many tumors are composed of a hierarchy of cell types at the apex of which lie so-called cancer stem cells (CSCs) that are highly tumorigenic and give rise to intermediate progenitors and terminally differentiated progeny. These CSCs are

therefore responsible for the sustained growth and cellular heterogeneity of tumors. Understanding how CSCs balance tumorigenic proliferation and differentiation is key for the development of future cancer therapies. Recent studies show that a robust homeostasis between cell type proportions in the tumor can be reached. How differentiation decisions are made during tumor growth to allow the emergence and maintenance of these features is unknown. Using simple brain tumors in Drosophila, we demonstrated that the overall tumor growth and composition is predictable and driven by a fine-tuned hierarchical division scheme. Using 3D simulations of tumor growth, we found that this tumor-averaged view is not sufficient to account for the observed segregation of the cell types. Using automated 3D segmentation of the different cell types, we analyzed the spatial organization of the tumor in vivo, and found that the observed tumor-scale features are actually supported by differentiation decisions taken locally. Most importantly, the probability to differentiate is directly imputable to the identity of the surrounding cells, and we were able to establish the differentiation probability as a direct function of a coarse-grained neighborhood index. Lastly, simulations and experiments suggest that the spatial segregation between CSC and their differentiated progeny is a tunable ingredient that confers homeostasis in cell type proportions and determines the CSC proportion. Overall, our results show that the balance between CSC self-renewal and differentiation is the result of local differentiation signals produced by cells along the CSC lineage, tempered by their ability to segregate.

Poster 23

Exploring evolutionary variations in forebrain development using patterned organoids

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The Francis Crick Institute

Vertebrate adult forebrains show a large variation in size and complexity across species despite the conserved nature of the regulatory molecules responsible for patterning. In the developing telencephalon, Shh (ventral) and Wnt/BMP (dorsal) signals induce the expression of region-specific transcription factors thereby dividing it into discrete zones of progenitor cells. The relative size and cell type composition of the progenitor zones influence the organization and complexity of the adult brain. The mechanism of how the differences in signalling activities of morphogens and their downstream regulation by gene regulatory networks translate into cell fate decisions of the early progenitor populations and therefore drive interspecies variation remains poorly understood. To tackle this gap in the field, I am establishing mouse and human organoid systems suitable for in vitro patterning. I have devised a protocol that generates single lumen telencephalic organoids from human and mouse embryonic stem cells and validated it. Now, I am probing the self-organising capacity of the system by exposing them to homogeneous morphogen concentration and then testing for emergence of dorsoventral pattern. I will be utilising cryogels and patterning chambers to impose anti-parallel gradients of ventral (Shh) and dorsal (Wnt) morphogens on the organoids in a controlled fashion to recapitulate the dorsoventral organisation of the nascent telencephalon. I will quantify cellular responses to morphogen input in the two species and analyse how spatiotemporal patterns change

in response to perturbations in morphogen dynamics. Using this experimental setup and mathematical modelling we aim to identify the species-specific properties of how cells and tissues respond to morphogen signals and subsequently determine the evolutionary differences in the developing telencephalon. Keywords: Organoids, morphogen, gene regulatory network, telencephalon dorsoventral patterning, evolution.

Poster 24

Dorsal morphogens in the regulation of Roof Plate size

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Morphogen signalling orchestrates tissue development by controlling the specification of cell identities as well as the rates of cell proliferation and cell loss. An excellent example of this is the dorsal neural tube. The roof plate forms the most dorsal cell population of the neural tube and is the signalling centre which regulates the growth and patterning of dorsal interneuron populations. The roof plate, along with neural crest, forms in response to BMP signalling from the surface ectoderm. The precise mechanisms by which these two populations form and how the size of the roof plate is regulated remains poorly understood. We developed a quantitative in vitro culture system that directs mouse embryonic stem cells to differentiate into dorsal spinal neural progenitor cells on micropatterns. This system allows us to precisely manipulate morphogen signalling dynamics and assess the cellular response. We are combining this approach with quantitative microscopy to measure changes in signalling. Preliminary data suggest the proportionality of these two populations are regulated by Bmp and Wnt signalling. Wnt signalling positively correlates with roof plate size and negatively correlates with the neural crest population size. On the other hand, Bmp signalling in the presence of Wnt inhibitors increases the neural crest population and decreases roof plate size.

Poster Session 2

Poster 25

Regulation of cell proliferation in regenerating zebrafish scales

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Cell proliferation needs to be precisely controlled for a regenerating body part to grow back to its original size and shape. Difficulties in visualizing cell signals and behaviours in vivo limited our understanding of how signalling pathways orchestrate cell proliferation in adult regenerating tissues. We investigate this question in regenerating zebrafish scales using live imaging and transgenic biosensors. Scales are discoid appendages including a bone plate deposited by an adjacent monolayer of osteoblasts. After scale loss, new osteoblasts form by differentiation and then proliferate for about two days. Thereafter, osteoblasts stop proliferating but continue growing in size (hypertrophy) driven by a series of travelling Erk activity waves. We

find that Erk is also required for osteoblast proliferation. Thus, we set out to characterize the Erk activation pattern using a live Erk activity sensor. Erk is initially uniformly active, then it switches off starting from the scale center; then, a band of high Erk activity forms at the scale boundary. Intriguingly, osteoblasts appear divided in two populations: a first population proliferates following Erk activation, a second one grows hypertrophically. Single-cell and bulk transcriptomics show that these two populations are, respectively, less and more mature osteoblasts. This suggests that osteoblasts progressively mature and lose proliferation competence as the proliferative phase proceeds. We find that mature osteoblasts appear centrally in the scale and progressively extend in the entire tissue, similarly to the pattern of Erk deactivation. Remarkably, the region of hypertrophy follows the growing edge of the nascent bone, thus suggesting that osteoblast-secreted bone matrix drives their maturation and Erk switch off. We are now using new transgenic markers of osteoblast maturation and perturbations to investigate this hypothesis. Overall, this project is revealing how the interplay of signals, bone formation and cell state controls different stages of appendage regeneration.

Poster 26

Dynamical systems theory of cell differentiation and reprogramming

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National Centre for Biological Sciences

Poster 27

Deformation of the embryo affects the direction of cell migration during anterior-posterior axis formation in mouse

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In mouse development, the anterior-posterior(A-P) axis specification depends on the migration of the cell population called the anterior visceral endoderm(AVE). AVE is known to induce the future anterior side of the body by inhibiting posteriorizing signals including Wnts. Thus, the direction of AVE migration plays a key role in determining the direction of the A-P axis. AVE migrates asymmetrically from the distal end to the proximal side of the embryo. However, the mechanisms that control the direction of AVE migration remain elusive. We have been trying to understand the mechanisms of how the embryonic A-P axis is determined in the uterus, focusing on the role of the uterus, which is an environment supporting embryonic development, especially on the mechanical influence to the embryo that may affect the migration of AVE. We found that the shape of the embryo in the transverse section was ellipsoidal in the uterus during AVE migration. AVE localized randomly within the ellipsoidal transverse section at the early stage of migration but, it localized at the end of the minor axis at the late stage of migration. To test whether AVE migrates toward the minor axis end when the

embryo is experimentally deformed by mechanical force exerted, we developed an ex-utero culture system combined with embryo compression. Without compression, the transverse section of the embryo was nearly circular and AVE migrated toward a part of it. On the other hand, when the embryo was compressed and the transverse section became ellipsoidal, AVE migrated toward its minor axis end. This suggests the unknown mechanism by which AVE senses mechanical force applied to the embryo and regulates the direction of its migration.

Poster 28

Cell volume patterning as a mechanism for mitotic phase waves in embryonic development

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ISTA

In many species, the initial embryonic cleavages occur synchronously but gradually transit into near (meta)synchrony and eventually asynchrony. Embryos in which synchrony is perturbed fail to develop, suggesting that cell cycle synchrony is likely of critical importance. Using the zebrafish embryo as a model, we are attempting to understand how the synchrony arises and is eventually lost. Cell cycles are synchronized through mainly two mechanisms: trigger waves (require cell-cell coupling) and phase waves (uncoupled internal clocks autonomously time cleavages). We show that zebrafish cells cycle together as their clocks are ‘in phase’. Gradually, they drift out of phase due to disproportionate S-phase lengthening away from the animal pole, producing mitotic waves originating at the animal pole. Preventing such ‘individual’ cell behavior prolongs metasynchrony, suggesting that in unperturbed embryos, coupling is minimal. Our theoretical model predicts that animal pole-origination requires coupling to be minimal. Indeed, in embryos with greater coupling, the wave originates at the margin. What causes patterned lengthening of cell periods? One aspect of cells that controls their periods is size; larger cells divide more frequently. Along these lines, our initial data show that cell size might indeed be patterned- cells at the animal pole are larger – and that altering cell size does, in fact, alter the kinetics of S-phase lengthening. Finally, as the loss of metasynchrony coincides with the onset of zygotic transcription, we are also investigating a potential interplay between cleavage desynchronization and fate specification/zygotic transcription asymmetries, which will provide key insights into the functional relevance of mitotic synchrony (and the eventual lack thereof) in embryonic development.

Poster 29

Unveiling a signaling role of metabolism in regulation of developmental timing in mammalian embryos

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EMBL Heidelberg

How metabolism impacts cell fate decisions during development and disease is a fundamental yet unresolved question. It is increasingly clear that metabolism impacts gene expression and signal transduction, while fueling biological processes by

providing energy and biomass. A key question was whether metabolism plays an instructive rather than a permissive role in regulation of cell fate decisions. In this meeting, we will present that glycolysis exerts an instructive function via developmental signaling, not via cellular bioenergetic state, to regulate the timing of mouse presomitic mesoderm (PSM) development (Miyazawa et al., 2024, bioRxiv, doi.org/10.1101/2024.01.22.576629). Vertebrate embryos undergo periodic segmentation of the PSM. This temporal periodicity is controlled by a molecular clock known as the segmentation clock, comprising of the Notch, Wnt, and FGF signaling pathways in mice. Since we previously revealed the necessity of active glycolysis for PSM development (Bulusu et al., 2017, PMID 28245920), our primary interest was to reveal whether and how glycolysis plays an instructive rather than a permissive role in this context. We first revealed that glycolytic flux tunes the segmentation clock period in an anti-correlated manner: increasing glycolytic flux slows down the clock, and vice versa. Transcriptome analysis identified the Wnt signaling pathway as a flux-responsive development signaling (Miyazawa et al., 2022, PMID 36469462), which potentially underlies the flux-dependent control of the clock period. To functionally challenge this possibility, we employed both genetics and entrainment-based dynamical systems approach, and found evidence showing that glycolytic flux acts through Wnt signaling, rather than cellular bioenergetic state, to regulate the clock period. Combined, our work demonstrates a glycolysis-Wnt signaling axis that tunes developmental timing, highlighting an instructive signaling role of metabolism in developing embryos.

Poster 30

Trajectory mutual information in biochemical systems: Gaussian vs. Poissonian fluctuations

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MPI-CBG/CSBD

Signal processing in biochemical networks relies on dynamic information transmission between time-trajectories of the respective molecular components. From a mathematical point of view, the transferred information can be described via the mutual information. Traditionally, this has been calculated using a Gaussian approximation. Our recent work suggests that this method is not always suitable for every biochemical networks which can lead to quantitative and qualitative mismatches to the exact solution. In this work, we explain the origin of these discrepancies and present a modified version of the Gaussian framework that aligns better with the characteristics of stochastic biochemical networks.

Poster 31

Single-nuclei sequencing reveals cellular heterogeneity and differentiation dynamics within the shoot apical meristem

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Sainsbury Laboratory Cambridge University

Unlike in many animals, where organs form only during embryogenesis or larval periods, plants continuously generate organs, producing new organs and cell types

throughout their lifespan. This process emanates from stem cells embedded in specialized regions knowns as meristems. The shoot apical meristem (SAM), located at the plant apex, is accountable for the formation of all above-ground organs such as leaves, stems and flowers. In angiosperms, the SAM comprises distinct regions of slowly dividing stem cells that will displace daughter cells outwards toward the periphery where they will differentiate into the various cell types observed in the above-ground tissue. Although transcriptional profiling has further elucidated some cell-types observed in stems or flowers, the mechanisms underlying the generation of this plethora of cell identities from stem cells located in the SAM has remained unknown. In this study, we employed a single-nucleus RNA-sequencing (snRNA-seq) approach to unravel the transcriptional heterogeneity and cell differentiation processes within the SAM. By collecting dissected inflorescence meristems from Arabidopsis thaliana, we constructed the first inflorescence single-nucleus SAM atlas comprising 7,295 valid cells. Our analysis unveiled regulatory elements for most previously known cell types such as boundary domain, vasculature, early primordia, epidermis and internal stem cells. Strikingly, we identified previously unobserved transcriptional profiles, revealing that the stem cortex is defined early from L2 internal stem cells. Moreover, trajectory inference analysis allowed us to capture the differentiation gene expression dynamics from internal shoot stem cells toward internal layers within the stem and flower such as xylem, phloem and cortex. In summary, our findings advance our understanding of the diverse cellular and transcriptional heterogeneity underlying the cell-fate transcriptional dynamic shaping above-ground organs and shoot architecture from shoot stem cells.

Poster 32

Keratins contribute to zebrafish epithelial morphogenesis

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ISTA

Intermediate filaments play central roles in protecting epithelial tissues from external forces and regulating their morphogenetic capacity. Yet, remarkably little is known about the mechanistic basis by which intermediate filaments function in these processes. Here we show that the keratin intermediate filaments function as a rheostat during epithelial cell layer spreading, ensuring highly coordinated and robust tissue expansion in response to external pulling forces. By analysing the expansion of the enveloping cell layer (EVL) in early zebrafish embryos in vivo, we found that keratin network maturation in EVL cells and the adjacent yolk cell, over which the EVL spreads, are promoted by tension building up within the plane of the spreading tissue. Tension-induced keratin network maturation in the EVL leads to increased tissue viscosity, while in the yolk cell, it promotes actomyosin network reorganisation and contraction, the force-generating process driving EVL spreading. Interfering with keratin expression leads to EVL fluidization and reduced actomyosin contraction within the yolk cell, which together slow down EVL epiboly movements. Therefore, keratins function as a rheostat balancing EVL viscosity, and thus its resistance against deformation, to the pulling force within the yolk cell driving its expansion, ensuring timely and robust tissue spreading.

Poster 33

Evolution of hierarchy and irreversibility in theoretical cellular differentiation model

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Multicellular organisms generate various cell types during the cell differentiation process. This process is characterized by two significant features, namely hierarchy and irreversibility. These features of cell differentiation are commonly seen in various species. Though many research works have proposed minimal theoretical models that can exhibit hierarchical and irreversible differentiation, the question of why such characteristics are commonly observed remains poorly understood. As differentiation diagrams are the outcome of evolution from simpler structures, the question should be understood through evolutionary process. More specifically, what conditions and selection pressures give rise to hierarchy and irreversibility? By what mechanisms are they acquired? To answer these questions, we employed an abstract cell model to explore how hierarchy and irreversibility emerge in cell differentiation during evolution. In this model, the regulation of cell states by gene regulatory networks (GRNs) is described as a simple dynamical system, whose attractors are considered as cell types. We further define differentiation as the transition between attractors as a result of perturbations to the expression state. In our study, these multicellular systems defined above were optimized to increase the number of terminally differentiated states, envisioning an abstract evolutionary process. We varied the magnitude of perturbations and found that high perturbation conditions resulted in inevitable hierarchical differentiation, whereas low perturbation conditions did not. Additionally, when the obtained differentiation diagram was hierarchical, it was also irreversible. These findings suggest that hierarchy and irreversibility are the inevitable outcomes of evolutionary process. We also have clarified the mechanism theoretically by analyzing the geometrical aspects of the cell state space. Lastly, we explored the typical structures observed in GRNs that result in differentiation diagrams displaying hierarchy and irreversibility. Our analysis has revealed a GRN structure that differs from the previously assumed bistable switch linkage, and we propose it as a new hypothesis.

Poster 34

Information transmission in a cell monolayer: A numerical approach

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Cells usually communicate with each other using local interactions, such as para-/juxtacrine signaling or mechanical strain. These local interactions can be used to tranfer information over a longer distance -- propagating waves of cell activation have been observed, e.g, in mammalian wound healing and scale formation in fish. I will present how we used the SEIR model to quantify the maximal rate of information that can be transferred by a cell monolayer using transient activity pulses. Based on the model, we found out that the information transfer is most efficient in cell populations

only several cells broad and that the cell activation rate must be much faster than recovery from the refractory period to make the information trasmission possible over a long distance.

Poster 35

Novel pattern propagation mechanism: Pattern propagation driven by surface curvature

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The University of Tokyo

Pattern formation and dynamics are highly organized in biological systems. Patterns appear abundantly on curved surfaces, and surface curvature is known to influence pattern dynamics and play biological roles. For example, Hydra regeneration is organized by the topological feature of actin fibers on the embryos's surface. However, the understanding of the relationship between patterns and surfaces is still developing. In this presentation, we report a novel mechanism by which pattern dynamics arise: surface curvature drives pattern propagation. Using a reaction-diffusion system exhibiting a Turing pattern, we show that a static pattern on a flat surface can propagate on a curved surface. In addition, both static and propagating patterns exist on curved surfaces, depending on the model parameters, surface geometry and initial conditions. Numerical and theoretical analyses reveal that, for general reaction-diffusion systems, the onset of pattern propagation depends on the symmetry of the surface and the pattern. This result indicates that the onset of pattern propagation can be controlled by surface topography.

Poster 36

Evolution of robust cell differentiation under epigenetic feedback

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Niels Bohr Institute

In multi-cellular organisms, cells differentiate to multiple types as they divide. Here these cell types as well as the developmental course is known to be robust to external perturbations, as conceptualized by Waddington's epigenetic landscape where cells embed themselves in valleys corresponding with final cell types. How is such robustness in developmental path, termed as homeorhesis, achieved by developmental dynamics and evolution? To address the question, we consider a model of splitting cells with gene expression dynamics and epigenetic feedback, governed by the gene regulation network. By evolving the network to achieve more cell types, we identified three basic mechanisms and expression dynamics to promote cell differentiation, that emerge depending on the noise level in the dynamics. Under noise, the gene expression dynamics first reach oscillatory dynamics described by low-dimensional orbit space, whereas sub-cycles are generated to make hierarchical differentiation. Higher noise levels however seem to avoid oscillatory dynamics but creates hierarchy through branched splitting to attractors. The final scenario seem to take a more intermediate approach while having orbits moving through parallel lines and planes. The three mechanisms differ in the nature of initial oscillatory dynamics and sub-cycle generation, which also lead to difference in their robustness to initial

perturbations, mutations and in their final cell type distribution. Relevance of initial attraction to the oscillatory state to robust development is noted, whereas evolution of multicellular development to achieve multiple cell types is discussed.

Poster 37

Differential entropy as an indicator of differentiation in the early mouse embryo

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Heterogeneity in gene expression among single cells is critical for cell differentiation. Stochastic cell-to-cell expression heterogeneity followed by signal reinforcement is necessary for the initiation of differentiation. In the pre-implantation mouse embryo, Inner Cell Mass (ICM) cells can differentiate into Epiblast (Epi) or Primitive Endoderm (PrE) cells. This asynchronous differentiation is driven by the activity of NANOG, GATA6 and ERK-mediated inter-cellular signaling. A key question is to identify genes that initiate and drive the coordination of pluripotency factors and specification of Epi cells alongside NANOG at the onset of differentiation. Here, we analyzed five single-cell transcriptomic datasets (RT-qPCR and RNA-seq) and suggest, through the analysis of the inter-cellular differential entropy, four possible candidate genes - Hnf4a, Lcp1, Pecam1 and Sox2 - whose coordinated heterogeneity alongside NANOG could drive the differentiation of ICM cells into the Epi cell fate. We also show that other typical measures of variability, such as the Fano factor or the coefficient of variation, show a less robust temporal profile compared to that of differential inter-cellular entropy and that the identification of the candidate genes would not have been possible with a standard analysis based on principal component and correlation analyses.

Poster 38

A genuinely spatial-stochastic model of early mouse embryogenesis via deep-learning simulation-based inference

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Understanding how multicellular organisms reliably orchestrate cell-fate decisions is a central challenge in developmental biology. This is particularly intriguing in early mammalian development, where early cell-lineage differentiation arises from processes that initially appear cell-autonomous but later materialize reliably at the tissue level. In this study, we develop a multi-scale, spatial-stochastic simulator of mouse embryogenesis, focusing on inner-cell mass (ICM) differentiation in the blastocyst stage. Our model features biophysically realistic regulatory interactions and accounts for the innate stochasticity of the biological processes driving cell-fate decisions at the cellular scale. We advance event-driven simulation techniques to incorporate relevant tissue-scale phenomena and integrate them with Simulation-Based Inference (SBI), building on a recent AI-based parameter learning method: the

Sequential Neural Posterior Estimation (SNPE) algorithm. Using this framework, we carry out a large-scale Bayesian inferential analysis and determine parameter sets that reproduce the experimentally observed system behavior. We elucidate how autocrine and paracrine feedbacks via the signaling protein FGF4 orchestrate the inherently stochastic expression of fate-specifying genes at the cellular level into reproducible ICM patterning at the tissue scale. This mechanism is remarkably independent of the system size. FGF4 not only ensures correct cell lineage ratios in the ICM, but also enhances its resilience to perturbations. Intriguingly, we find that high variability in intracellular initial conditions does not compromise, but rather can enhance the accuracy and precision of tissue-level dynamics. Our work provides a genuinely spatial-stochastic description of the biochemical processes driving ICM differentiation and the necessary conditions under which it can proceed robustly.

Poster 39

Role of tristability in the robustness of the differentiation mechanism

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During cell differentiation, identical pluripotent cells undergo a specification process marked by changes in the expression of key genes, regulated by transcription factors that can inhibit the transcription of a competing gene or activate their own transcription. This specification is orchestrated by gene regulatory networks (GRNs), encompassing transcription factors, biochemical reactions, and signalling cascades. Mathematical models for these GRNs have been proposed in various contexts, with the aim to replicate observed robustness in differentiation properties. This includes reproducible proportions of differentiated cells with respect to parametric or stochastic noise and the avoidance of transitions between differentiated states. Understanding the GRN components controlling these features is crucial. In our study, we thoroughly explored an extended version of the Toggle Switch model with auto-activation loops. This model represents cells evolving from common progenitors in one out of two fates (A or B, bistable regime) or, additionally, remaining in their progenitor state (C, tristable regime). Such a differentiation into populations with three distinct cell fates is observed during blastocyst formation in mammals, where inner cell mass cells can remain in that state or differentiate into epiblast cells or primitive endoderm. Systematic analysis revealed that the existence of a stable non-differentiated state significantly impacts the GRN's robustness against parametric variations and stochastic noise. This state reduces the sensitivity of cell populations to parameters controlling key gene expression asymmetry and prevents cells from making transitions after acquiring a new identity. Stochastic noise enhances robustness by decreasing sensitivity to initial expression levels and by facilitating irreversible transitions from the non-differentiated state to differentiated cell fates.

Poster 40

Self-organised pattern formation in the developing dorsal neural tube by a temporal relay of BMP signalling

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ISTA

In the vertebrate dorsal neural tube, neural crest, roof plate and dorsal neural progenitor subtypes are established in defined spatiotemporal order. To quantitatively study the underlying mechanism, we developed a new ES cell differentiation system based on stencil micropatterning tailored for growing tissues. We found that pattern formation in the dorsal neural tube has an intrinsic propensity to self-organise, and this depends on cell-intrinsic sequential phases of BMP signalling that are observed both in vitro and in vivo. Using time-resolved inhibition and rescue experiments together with biophysical modelling, we found that these biphasic dynamics result from coupling fast negative regulation and slow positive regulation of BMP signalling by roof plate specification and endogenous BMP ligand expression. This way, our data illustrate how the intrinsic dynamics of BMP signalling coupled with cell fate specification leads to self-organised patterning.

Poster 41

Understanding cell fate specification and maintenance through giant cell formation in Arabidopsis thaliana sepals

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How cell fate decisions are made through complex gene regulatory networks (GRNs) has been an important field of study. However, how these GRNs persist and maintain differentiated cell status has been less studied, even though this is crucial to the physiology and survival of living organisms as they develop. In the Arabidopsis thaliana sepal epidermis, there are two main cell types: small cells and giant cells. Giant cells endoreduplicate, i.e., skip mitosis, duplicate their chromosomes multiple times, and grow to large sizes. We have previously proposed a cell-autonomous, stochastic mechanism for giant cell fate specification, in which a transcription factor, ATML1, upregulates a cyclin-dependent kinase inhibitor, LGO, which in turn specifies giant cell identity. How ATML1 and LGO may be involved in maintaining the giant cell fate identity after giant cell fate specification was unknown. In this work, by using advanced experimental and imaging techniques, quantitative image analysis, mathematical modeling, and numerical simulations, we propose that a double positive feedback loop on ATML1 is crucial for the cell fate specification process, and it is also responsible for maintaining the giant cell fate. Specifically, we show that high concentrations of ATML1 induce the biosynthesis of its own (very) long-chain fatty acids, and these fatty acids are required for the maintenance of giant cell identity. Inhibition of fatty acid biosynthesis causes some endoreduplicated giant cells to lose their identity and resume division, indicating that endoreduplication is not sufficient to maintain cell identity. Based on our experimental findings, we constructed an analytical and stochastic computational model of giant cell fate decision-making in a growing and dividing tissue, recapitulating our experiments showing giant cell de-differentiation. Our work mechanistically provides a clear example of how a given regulatory circuit can operate for both the cell fate decision-making process and cell fate maintenance.

Poster 42

Transcription-dependent genome folding

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Although our understanding of the involvement of heterochromatin architectural factors in shaping nuclear organization is improving, there is still ongoing debate regarding the role of active genes in this process. In this study, we utilize publicly-available Micro-C data from mouse embryonic stem cells to investigate the relationship between gene transcription and 3D gene folding. Our analysis uncovers a nonmonotonic - globally positive - correlation between intragenic contact density and Pol II occupancy, independent of cohesin-based loop extrusion. Through the development of a biophysical model integrating the role of transcription dynamics within a polymer model of chromosome organization, we demonstrate that Pol II-mediated attractive interactions with limited valency between transcribed regions yield quantitative predictions consistent with chromosome-conformation-capture and live-imaging experiments. Our work provides compelling evidence that transcriptional activity shapes the 4D genome through Pol II-mediated micro-compartmentalization.

Poster 43

Role of biomechanics in the regulation of tissue growth during neural tube development

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ISTA

During development, cell proliferation and terminal differentiation are tightly balanced to ensure the correct organ size. One way to regulate the rates of cell proliferation and cell cycle exit is through mechanical forces. However, the role of mechanics in regulating growth in many tissues is still unclear. In the mouse neural tube, the motor neuron progenitors (pMN) exit the cell cycle at higher rates than neighboring domains at E9.5 of development. We found that this specific growth dynamics is accompanied by specific changes in the apical surface areas of cells that suggest altered line tension and contractility of pMN progenitors. To understand how the differentiation rate is related to changes in mechanical forces within the pMN domain, I measured the tissue tension in the pMN domain using laser ablation experiments. I found that the tissue tension in the pMN domain is higher during differentiation peak (E10.5) compared to a day later (E11.5), where differentiation rate has already declined. I hypothesize that this change in mechanics promotes the decline and subsequent cessation of pMN differentiation. To test the hypothesis, I am currently perturbing tissue tension and analyzing its effects on pMN differentiation. Preliminary data show that decreasing and increasing the tissue tension results in changes in the number of MNs and the pMN domain size. Overall, the data so far suggest that mechanical forces play a role in controlling growth and pattern formation in the developing neural tube.

Poster 44

Investigating the role of physical signals during vascular cells differentiation in

plants

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A particular difference regarding plants and animals developmental biology is that plants acquire their general form post-embryonically, keeping an open growth strategy. In plants, positional information plays a larger role in cell differentiation than cell lineage, especially since there is no cell migration and the cells are 'restricted' within a rigid extracellular matrix, the plant cell wall. Important aspects of hormonal and transcriptional regulation of cell fate in plant tissues are much clearer than the role of topology and physical signals. We address this understudied subject using a cell culture system in which differentiation is induced by a combination of hormones. In this case, a fraction of the undifferentiated cells differentiate into xylem (water conducting) cells. Xylem cell differentiation normally takes place inside the plant body under mechanical constrains and in a pre-patterned cell topology which arises from the surrounding tissues and has been hypothesized to contribute to cell fate acquisition. In our system, we can uncouple the above properties by working with cell cultures where cells arrange in seemingly random cell clusters. Since only some of the cells differentiate, we first investigate if during the hormonal induction, there is a mechano-sensing dependent positional information integrated. The information could be derived from an emerging cell arrangement in the cell culture, leading to the observed heterogeneous outcome of cell differentiation. Using confocal microscopy to track cell shape and fate visualised with fluorescent reporters and dyes, we aim to identify the presence of physical information that correlate with cell fate acquisition. Then, we will further assess the contribution of physical signals and topology modulation through external compression. With this work we aim to unveil how plants cells use physical information to guide their fate in vitro and later expend this knowledge to in situ cell differentiation processes.

Poster 45

A workflow to observe single-cell morphogenetic features of developing mucociliary epidermis

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As the developing embryo forms, the simple mass of cells becomes more and more complex throughout the morphogenetic shaping of tissues. Morphogenetic processes display an astounding level of tissue self-organization, where an initially unorganized mass of cells rearranges to form a functional tissue. One example of such process is the formation of regularly patterned, multilayered tissue, such as mucociliary epithelia (MCE); however, the formation of this tissue from initially pluripotent cells remains uncharacterized. The morphogenetic shaping and cell fate choices in the MCE takes place both collectively and individually. Cells exhibit collective movement, but single cells in in the deep cell layer can also migrate individually and remodel their environment. To resolve the morphogenetic behaviors across time and across the

scale of individualism-collectivity, we quantify morphological and kinetic phenotypes of single cells in the embryonic frog (Xenopus laevis) MCE. Using explanted prospective MCE, we can image and quantify developmental dynamics in single-cell resolution. To achieve this, we have developed a state of the art quantitative imaging pipeline to track cell dynamics in the bottom layer of developing Xenopus epidermal explants. The detailed backtracking of the cell's histories allows us to connect individual cell features to formation of collective behaviors that arise in the tissue over developmental time. By assaying the embryonic epidermis, we aim to provide an unprecedented detailed view of the developmental dynamics of a mucociliary epithelium. Understanding these fundamentals of mucociliary differentiation could provide a better understanding of pathological conditions arising from defective development of airway epithelia.

Poster 46

Uncovering rules governing self-organization of gastruloid morphology

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A characteristic of developmental systems is their ability to organize into a spatially-structured assembly of distinct cell types. While much research into self-organization, particularly in the context of embryogenesis, focuses on the ability of systems to form highly-reproducible structures, their morphologies nevertheless remain heterogeneous. Our work focuses on the gastruloid model of pre-implantation development. Despite recapitulating broad rules of germ-layer and body axis establishment, gastruloids display spontaneous emergence of variation in several morphological characteristics even under controlled conditions. The extent to which cell-extrinsic (e.g. morphogen gradients and relative cell position) or intrinsic (e.g. gene expression or lineage) factors dictate the spatial arrangements of cell types in the developing gastruloid therefore remains unclear. Here, we build a latent space of gastruloid morphologies to provide a quantitative description of their heterogeneity. Using fluorescent reporters for gene expression and cell lineage, we track cells through gastruloid development to uncover rules that dictate gastruloid morphology and connect individual cells' behaviors to a gastruloid's body plan. We find gastruloid morphology to be closely associated to the proportion of cells expressing the mesodermal marker gene T, a target of Wnt signaling, suggesting that cell-intrinsic differences in Wnt signaling contribute to morphological variability in gastruloids. Chemical modulation of Wnt signaling, and consequently of T expression, can reverse elongation, potentially by altering the level of cohesion among populations of cells in a gastruloid. Beyond expression of signaling pathway components, we find that distinct "Brainbow" lineages cluster to different regions of the gastruloid, even in gastruloids that have been dissociated and re-aggregated. Thus, lineage appears to influence how cells arrange themselves in a gastruloid and may be the basis for another local rule guiding gastruloid tissue structure. Ultimately, this work hopes to uncover limits on the ability of self-organizing systems to produce and maintain order.

Poster 47

Finding signatures of low-dimensional geometric landscapes in high-

dimensional cell fate transitions
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In many animals, hundreds of highly specialized cell types work together to maintain homeostasis. When growing or injured, cells can self-organize and transition between these cell types. The consistency and robustness of developmental cell fate trajectories suggests that complex gene regulatory networks effectively act as low-dimensional cell fate landscapes. While there has been progress in characterizing classes of cell fate decisions using gradient-like dynamical systems, the theory connecting geometric landscapes to high-dimensional gene expression space is still in its infancy. In this paper, we introduce a phenomenological model of cell fate transitions that predicts bifurcation signatures observable in gene expression measurements. By combining low-dimensional gradient dynamical systems and high-dimensional Hopfield networks, our model captures the interplay between cell fate, gene expression, and signals. The signal-driven bifurcations of an input landscape control the stability of attractors, and this feature allows the model to predict dynamics resulting from any class of bifurcation. Using existing single-cell RNA-sequencing time-series data, we compare experimental observations to theoretical landscape candidates belonging to different bifurcation classes. In the developing mouse lung, the transient appearance of a mixed alveolar type 1/type 2 state in the growing mouse suggests the maturation of alveolar cells is a triple cusp bifurcation. Additionally, previous analysis of lineage-tracing data of in vitro hematopoetic differentiation indicated that monocytes have a neutrophil-like path of differentiation; when compared to possible landscapes, bipotent neutrophil-monocyte progenitors appear to undergo a heteroclinic flip bifurcation. These results show that a geometric landscape approach can reveal new insights in time series single-cell RNA-sequencing data of cell fate transitions.

Poster 48

Developmental patterning and cell-fate specification of the mouse colonic epithelium
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The mammalian intestine transitions from a smooth tubular epithelium into a highly-ordered structure during development. Work in the mouse small intestine has found evidence for the essential role of both mechanical and biochemical cues in driving the self-organisation and patterning of the epithelium into the hallmark structures of crypts and villi during late embryonic and early postnatal development. Yet, the timing and mechanism of the formation of structures in the colon remain underexplored. Here, we focus on the origin and timing of the larger-scale epithelial folding and smaller-scale glandular organisation during colonic development; whether this organisation emerges deterministically or stochastically and whether developmental changes at different scales affect the molecular identities and fates of the cells making up the developmental epithelium. Combining quantitative imaging, fate mapping, ex-vivo live-

imaging, single-cell molecular profiling and biophysical modelling in the developing mouse colon, our results show how region-specific tissue-level remodelling restricts the initiation and expansion of the glandular organization. Timelapse imaging of ex-vivo culture experiments show strong evidence for distinct mechanically- and chemically-driven symmetry-breaking mechanisms across scales in the colon when compared to the small intestine. Quantitative lineage-tracing showed characteristic statistical distributions pointing to a stochastic growth pattern for the expansion of glandular organisation in the colon postnatally. Finally, statistical analysis of scRNAseq data show how colon regionalisation is correlated with fate decision timing, clonal composition, and molecular identity of cells in the developing epithelium. Together, these findings elucidate the physical and molecular events that drive the patterning of the colonic epithelium.

Poster 49

Correlation Information and Non-local decoding in development

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During development, cell identities dictated by their spatial positions is determined with astonishing precision and reproducibility levels. Previous work by Tkacik and others developed the concept of positional information in the formal framework of information theory, that allowed the connection between the variability in gene expression levels in the developmental ensemble and the precision attainable by cells during their fate specification. Recent work by Brückner et al and McGough et al studied the previously neglected aspect of spatial correlations and their important role in maximizing reproducibility. In our work we study the information contained in the spatial correlations of gene expression levels within the embryo, and we show how this information could theoretically be exploited by cells. In particular we demonstrate that for inferring or decoding their positions, in presence of spatial correlations of a morphogen profile, cells can benefit from using not only their local readout of morphogen concentration but also non-local-information coming from readouts of their neighbors.