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LABEX WHO AM I?

EXPLORING IDENTITY: FROM MOLECULES TO INDIVIDUALS

ABSTRACTS

ENSA PVS auditorium
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FOCAL ADHESIONS ARE SEGREGATION SITES FOR GROWTH FACTOR RECEPTORS

Corinne ALBIGES-RIZO

The spatial organization of cell-surface receptors is fundamental for the coordination of biological responses to the multitude of physical and biochemical cues of the extracellular matrix. Many examples demonstrate the intimate crosstalk between the functioning of growth factor receptors and integrin signaling. Integrins are known to cluster in focal adhesion upon engagement with the extracellular matrix ligands. BMP2 signaling requires a complex of type-I (ALK3) and type-II (BMPRII) serine/threonine kinase receptors to activate the canonical and non-canonical SMAD signaling pathway. We have revisited this crosstalk in different mechanical environments to have a better understanding of the tuning of integrin signaling by growth factor. First, we have shown that in response to BMP2, a complex of type-I (ALK3) and type-II (BMPRII) serine/threonine kinase receptors cooperate with integrins to drive cell spreading and migration. We have also explored whether the spatial arrangement or dynamics between integrin and BMPR at cell surface is controlled in space and time to guide pivotal intracellular processes. Using state-of-the art tools and approaches including fluorescent receptors, optogenetic, single-protein tracking, super-resolution imaging, and BMP2 presenting-biomaterials with controlled stiffness, we evidenced that upon BMP2 stimulation ALK3 and BMPRII are discretely organized to segregated spatial domains at the cell surface. BMP2 elicits the selective recruitment of ALK3 and not BMPRII into integrin-containing focal adhesions. ALK3 partitioning within and outside focal adhesion reveals the potential of two differentially regulated populations of ALK3 receptors. We show that the segregation of ALK3 into integrin-containing focal adhesions is a key aspect of the spatio-temporal control of a non-canonical BMPR signaling to drive cell adhesion and migration processes. The partitioning of BMPR points out the benefits of regulating the multimerization of receptors at cell membrane.

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BRINGING CHANCE BACK INTO BIOLOGICAL PROCESSES. DISSECTING STOCHASTICITY IN THE TIMING OF TRANSLATION

Marco CASALI

Chance in gene expression is typically addressed as “noise” in the context of transcription. By contrast, since translation is usually seen as “a linear conversion with one predictable, unambiguous outcome” (Putois et al 2015), its relation with stochasticity is seldom addressed. I have already explored the role of chance in transcription (Casali and Merlin 2020) arguing that it can be more than just noise, i.e. chance is an epistemic element having a specific role in biological explanations. In my presentation, I want to extend this kind of analysis to translation. Since transcription and translation processes happen in similar physico-chemical conditions, there is no a priori reason, theoretically speaking, that stochasticity would not affect this other stage of gene expression. This finds empirical confirmation in recent experimental research that clearly shows the pervasive influence of stochasticity on translation (e.g., Boersma et al 2019). Furthermore, in situations of cellular stress, translation privileges the synthesis of alternative proteins rather than standard ones (Sriram et al 2018). This event, called alternative start-codon selection, has recently been qualified as stochastic (Boersma et al 2019). Alternative proteins synthetized by chance are often seen as “error[s] in translation” (Boersma et al 2019, p. 459) or “translational noise” (Pauli et al 2015, p. 6). By contrast, I will ask whether the production of alternative proteins could be considered as a sort of bet-hedging strategy enacted by cells in order to face environmental stresses. If, following Orr et al (2020), these proteins can have a role in tumor prevention and in the maintenance of cellular homeostasis, then it seems they are essential for cell integrity. If so, we should re-examine intracellular processes at their origin from this perspective, and in particular re-evaluate the possible explanatory role of their stochastic character.

FLUID PUMPING AND ACTIVE FLEXOELECTRICITY CAN PROMOTE LUMEN NUCLEATION IN CELL ASSEMBLIES

Charlie DUCLUT

The presence of fluid-filled cavities (or lumen) is a common feature of multicellular structures. Nucleation and growth of such lumens typically involve the pumping of fluid by cells, which relies on active ion transport. In this talk, we will discuss a continuum description of a spherical cell assembly that takes into account fluid pumping, electric currents, and electric fields to explore the physical mechanisms of lumen formation. We highlight the role of the coupling between tissue bending and electric fields, called tissue flexoelectricity, in the lumen nucleation process. We show, in particular, that understanding lumen formation requires to consider the combined effects of mechanical, electrical, and hydraulic mechanisms.

MINIBAR: A DUAL RAB AND RAC EFFECTOR THAT CONTROLS CILIA LENGTH AND LEFT-RIGHT ASYMMETRY IN VIVO

Arnaud ECHARD

Primary and motile cilia are microtubule-based organelles that protrude from the cell surface and play critical roles in signaling and embryonic development. Cilia malfunction can lead to a group of diseases known as ciliopathies, which can result in various pathologies including polycystic kidneys and heterotaxy. Proper actin-dependent cell contractility and intracellular trafficking are both required for ciliogenesis, but little is known on how these processes might be coordinated. I will present the characterization of a protein displaying an unusual truncated BAR domain that we named MiniBAR, as a Rac1 and Rab35 binding protein important for ciliogenesis. MiniBAR colocalizes with Rac1 and Rab35 at the plasma membrane and on intracellular tubulo-vesicles trafficking to the ciliary base, and exhibits previously unseen fast pulses at the ciliary membrane. MiniBAR depletion results in both abnormal Rac-GTP/Rho-GTP levels, increased acto-myosin-II-dependent contractility, and defective trafficking of IFT88 and ARL13B into cilia. Consequently, zebrafish embryos depleted of MiniBAR display short, unfunctional cilia and defects characteristic of ciliopathy. Thus, MiniBAR represents a unique dual Rac and Rab effector, and controls both actin cytoskeleton and membrane trafficking for successful ciliogenesis.

REVISITING ENHANCER MODULARITY AND EVOLUTION IN *DROSOPHILA*

Nicolas GOMPEL

The diversification of morphological traits entails changes in size, changes in shape, as well as extensive quantitative variation of other phenotypic dimensions, such as color or texture. These changes often find their origin in gene regulation, particularly at the level of transcriptional enhancers. To understand how enhancers accommodate evolutionary changes underlying morphological diversity, we use various species of *Drosophila* with diverse patterns of pigmentation on their wings. We examine how transcriptional enhancers of a pigmentation gene involved in this variation emerge and diversify to tune pigmentation patterns. This leads us to integrate different levels of biological complexity, from atomic interactions between DNA and transcription factors, to the enhancer chromatin accessibility and its quantitative spatial output in developing tissue, and to the resulting pigmentation on fly wings. With a first project, I will show how our quantitative approach led us to reconsider the concept of enhancers as discrete and modular elements. With a second project, I will illustrate how the evolutionary tuning of the activity of a single enhancer has produced a continuum of the variation in wing pigmentation intensity between species.

INVOLVEMENT OF THE MFD PROTEIN IN SPONTANEOUS MUTAGENESIS IN *ESCHERICHIA COLI*

Flavia HASENAUER

The mutation frequency decline (Mfd) protein plays a fundamental role in the transcription-coupled repair pathway. Mfd is responsible for the displacement of RNA polymerase stalled by DNA damages which is followed by the recruitment of nucleotide excision repair (NER) machinery. NER starts with the recognition of the damaged area by the UvrA protein, which is followed by the excision of the damaged area by other NER proteins and is finalized by DNA polymerase that fills the resulting gap. While much is known about the involvement of Mfd protein in the repair of exogenously induced DNA damages, little is known about spontaneous mutagenesis.
To detect mutations, we have developed a mutation reporter system based on the mismatch repair system (MMR) which allows visualization and quantification by microscopy of the appearance of mutations in living individual cells using fluorescent MutL protein (Woo et al., 2018).
As the first part of our project, strains deleted for our candidate genes *mfd* and *uvrA* were constructed and the rate of spontaneous mutations was estimated using the MMR reporter system. In cells growing under non-stressfully conditions, we found fewer mutations in the *Δmfd* and *ΔuvrA* strains compared to the control. These observations suggested that proteins encoded by *mfd* and *uvrA* genes promote spontaneous mutations. Because both mutant strains showed very similar reduction of mutation rates, it is plausible that they act in the same pathway. To further characterize molecular mechanisms involved in the Mdf-associated mutator phenotype, we will also identify where these mutations appear in the genome by using chromatin immunoprecipitation (Chip-Seq) of the MutL protein.

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PLASTICITY OF THE CELLULAR IDENTITY AT THE UNIQUE CELL LEVEL

Sophie JARRIAULT

In some organisms, such as the microscopic worm *C. elegans*, some cells completely change their identity during development. This process is called reprogramming, and the cell will then have a completely different function. This is the case, for example, of a cell of the rectum which leaves this organ and becomes a motor neuron. The worm being transparent, we study and compare different natural reprogramming events at the single cell level to understand how they are controlled, how the presence of cell division can impact and what is the cellular trajectory taken during the transition between two identities.

HUMAN NATURE IS HUMEAN NATURE

Tim LEWENS

In this talk I outline and defend a simple account of human nature that derives from the writings of David Hume. Human nature, according to this Humean view, consists in the most widely shared, and most ‘deeply radicated’, characteristics of members of the human species. I contrast this position with alternative proposals that come from Edouard Machery and Grant Ramsey, and I suggest that the simple Humean account accords better with some very recent scientific work, especially with work that highlights the importance of phenomena such as niche construction, cultural evolution and epigenetic inheritance. This account consequently offers a middle way in recent disputes between those scientists and philosophers who are sceptical of the very idea of human nature, and those who think that medicine and the sciences—especially the sciences of human cognition and human behaviour—cannot get by without the notion. The account also explains why the notion of human nature has been so controversial, and why it is rightly thought to do damage in many contexts. The Humean account is, however, exceptionally modest in terms of what it gives by way of guidance in debates around the ethics of technologies that are often thought to modify our shared ‘nature’.

BIOLOGICAL IDENTITY AND PERSONAL IDENTITY: A PROCESS VIEW

Anne Sophie MEINCKE

Biological identity – the question of what it is that constitutes a biological individual at a time and over time – is a hot topic in today's philosophy of biology. Personal identity – the question of what it takes for a person to persist over time – has been a hot topic in metaphysics for at least four centuries. In this talk, I discuss the importance of the concept of biological identity for understanding personal identity and the role it plays in corresponding debates in metaphysics. Currently, a growing number of metaphysicians – so-called animalists – recognise the biological nature of human persons and account for personal identity in terms of biological identity. However, these animalist theories tend to ignore (or cover up) the various challenges that the question of biological identity in fact poses, as revealed by debates in the philosophy of biology. I argue that meeting those challenges requires a fundamental change in ontological commitments: organisms must be understood not as substances or things (as has traditionally been the case) but as processes. Only a processual animalism can deliver a scientifically sound account of biological and thus personal identity.

THE REPRODUCTION OF IDENTITY

Laura NUNO DE LA ROSA

The development and reproduction of biological identity is a major topic of concern in contemporary evolutionary biology. In the neo-Darwinian perspective of evolution, the reproduction of biological identity was conceived of in terms of replication of genetic information, and variations of identity relationships as resulting from random changes in the process of replication. In the last decades, this reduction of reproduction to replication has been challenged on several fronts. Evo-devo has shown that genetic identity cannot fully account for the identity of characters at a morphological and physiological level, for which mechanisms at a developmental level need to be unraveled. Niche construction theory as well as extended theories of inheritance have argued that non-genetic factors, such as epigenetic factors or engineered environments need to be transmitted across generations in order to reproduce phenotypic identity. In this talk, I will focus on a new emerging challenge to the replicator framework which concerns the ways in which reproductive systems actively engage in the reproduction of general and specific biological identities. As a case study, I will focus on the case of eutherian pregnancy to discuss how different mechanisms of maternal selection (including gamete and oocyte selection) participate in the recognition of identity relationships and contribute to the generation of the organismal identity of offspring. In doing so, I will discuss several identity criteria used by contemporary developmental and reproductive biology.

INTRAGENIC CPG ISLAND ACTIVITY INFLUENCES ALTERNATIVE SPLICING AND POLYADENYLATION GENOME-WIDE

Rebecca OAKEY

A quarter of mammalian genes exist as pairs where a transcriptional unit is embedded within another one. These are known as host/nested gene pairs. The regulation of host/nested gene pairs must overcome the challenges of the transcription, splicing and polyadenylation machineries occurring at the same time but at different genes that occupy the same genetic space. CpG islands found within host genes or intragenic CpG islands (iCGIs), can serve as promoters for these nested genes. iCGI activity is correlated with premature transcript termination, likely through alternative polyadenylation (APA) mechanisms and is mediated by epigenetics factors. This is the case at the host/nested gene pair, *H13/Mcts2*. This pair of genes are imprinted and provide a model system for examining the active and silent alleles in the same cell and genetic context. The active copy of *Mcts2* results in intronic polyadenylation, as opposed to canonical 3'UTR polyadenylation, of the *H13* host transcripts indicating that host/nested gene pair crosstalk can favour APA. To understand the mechanisms governing these APA events, we are using two approaches, a) a reporter system to allelically tag the products of alternative polyadenylation and exposing them to a CRISPR knock-out screen and b) CLASP (Cas9 locus-associated proteome) to identify novel regulators of APA. These experiments aim to highlight factors in the regulation of APA at host/nested gene pairs which we expect will be fundamental for cellular function, in particular, during differentiation and development.

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NUCLEAR PORE-ASSOCIATED TRANSLATION AND THE MAINTENANCE OF NUCLEAR IDENTITY

Vincent PACINI

In eukaryotic cells, a number of cellular domains or compartments display specific molecular contents, thereby accomplishing distinct biochemical functions. Asymmetrical mRNA localization, by enabling site-specific translation, has emerged as a universal strategy to target proteins to a given location in the cell, thus defining the specific proteome of intracellular compartments. As a defining feature of eukaryotes, the nucleus also displays a unique molecular composition devoted to the accomplishment of genomic transactions. Although proteins were believed to enter the nucleus through nuclear pore complexes (NPCs) in a post-translational manner, the lab recently reported the identification of a subset of mRNAs translated at the vicinity of the nuclear envelope in the yeast S. cerevisiae. Localized translation involves the recognition of nascent polypeptides by karyopherins and their subsequent association with NPCs, likely allowing co-translational import. The aim of this project was thereby to expand the repertory of mRNAs translated at NPCs, to get insight into the associated mechanisms and to decipher the regulations targeting this process. Through RNA immunoprecipitation coupled to high-throughput sequencing (RIP-seq), we identified hundreds of mRNAs specifically copurifying with isolated NPCs scaffolds. By combining genetics and cell imaging, we were further able to characterize the mechanisms and the functional relevance of localized translation for a representative NPC-associated mRNA. Finally, we found that the NPC-localized mRNA repertory is submitted to modulation in response to genotoxic stress. Localized mRNA translation at nuclear pores may thus fine-tune the identity of the nucleus, and respond to multiple physiological, stress or pathological situations impacting its content.

SHAPE AND SIZE OF THE CELL AND ITS NUCLEUS DURING GROWTH AND MIGRATION

Matthieu PIEL

Cell shape changes can impose various regimes of mechanical constrains on the nucleus depending on its degree of deformation. The mechanical state of the nucleus relies on two main components: the mechanics of the nuclear envelope (NE) and the osmotic equilibrium between the cytoplasm and the nucleus. In a first range of deformation, folds of the NE ensure a deformation at constant volume and low surface tension. Once the folds are open, there is an abrupt transition to a regime in which deformation translates into increased NE tension and internal nuclear pressure, leading to nuclear volume changes, activation of specific signaling pathways and an increase in the frequency of nuclear blebs and NE ruptures. I will present two very different contexts in which an increase in NE tension has important biological consequences for immune and cancer cells: 1) the coupling between the size and shape of single proliferating cells and their progression through the cell division cycle; 2) the activation of immune cells by massaging of their nucleus as they migrate through dense tissues. In conclusion, I will show how the various mechanical regimes of nuclear deformations, explained by simple physical laws, dictate distinct behaviors and fates in immune and cancer cells.

HAVING FUN WITH EPITHELIUM AND SPHEROIDS

Jacques PROST

After introducing the notion of homeostatic pressure, I will subsequently introduce dynamical equations, which exhibit fluid like behavior on time scales long compared to duplication and apoptosis times, in the vicinity of homeostatic conditions. Subsequently, I will describe stress-clamp experiments, which provide numbers on the effects of stress on cell division and apoptosis and introduce the idea of “active” tissue. Then I will describe a dynamical transition in nematic epithelia which we predicted more than ten years ago in the context of active gels: a nematic epithelial tissue placed on stripes of different width, switches from a perfectly quiescent state to a spontaneously shearing state, simply by changing the stripe width! Subsequently, I will give predictions about long time electric effects on polar tissues both for thick epitheliums and tissue spheroids. In particular I will show that simple symmetry-based arguments allow to predict field induced proliferation and death of thick epithelial tissues without mutations, as well as lumen formation in spheroids. In this last case a phenomenon called flexoelectricity is predicted to potentially play a significant role. Last, I will show a few striking experimental results concerning monolayer epitheliums submitted to a modest external electric field.

SYSTEMATIC PRE-SYMPTOMATIC SCREENING OF HEREDITARY PATHOLOGIES: ETHICAL AND PSYCHOLOGICAL ISSUES AND CONSTRUCTION OF COLLECTIVE REFLECTIONS

Anne-Laure SEBERT

Our postdoctoral research is being conducted within Necker Hospital rare metabolic diseases department. It is framed by an extension of neonatal testing to include seven new diseases as from January 2023.
In this statement, we will focus on the impact of this new data on medical and nursing practice as well as on patient management, in a "translational" research perspective. The extension of testing opens up specific ethical issues for field teams. Indeed, the heterogeneous sensitivity of the tests can result in "false-positives" which could lead to over-medicalization of healthy patients or patients with minor & benign form of the disease. This could impact the parent-child relationship.
Organizational challenges will also have to be overcome as new screenings will drag new patients within the health care system (patients suffering from severe or partial forms of the disease; siblings being tested; collateral diagnoses) in a context of unprecedented bed shortage.
By upsetting the classical medical temporality (symptoms, announcement, hospitalization) these tests impact the professional identity of the involved practitioners. Moreover, delivered information grows in complexity as new diseases screening are being included. As a result, we reckon that parents could refuse to test their child. This is why the arrival of these new testing should be considered as a major public health challenge.

PERTURBATIONS IN MUSCLE STEM AND NICHE CELL DYNAMICS IN PATHOLOGIES

Shahragim TAJBAKHSH

Viral infections, such as the flu, induce a host immune response to combat pathogens, which are associated with systemic inflammation. Influenza virus infects primarily the respiratory tract and not skeletal muscle, yet it induces secondary symptoms such as muscle weakness, which has severe consequences in populations with preconditions. Our study focuses on the indirect effects of viral infections on muscle stem cells (MuSCs) exposed to systemic inflammation following intranasal infection of mice.
We show that MuSCs respond to the distal influenza viral infection and alter their properties including a reduced size in vivo. Using EdU pulses in vivo and in vitro to monitor cell proliferation, we show that MuSCs are impaired in their ability to enter the cell cycle. Surprisingly, regulators or markers of quiescence, such as HeyL, Calcitonin receptor, and Pax7 are downregulated, but commitment and differentiation are not induced as shown by the absence of Myod protein. Further, MuSCs from influenza virus infected mice have a reduced metabolism during homeostasis. These alterations result in delayed and compromised muscle regeneration following injury. We propose that MuSCs exposed to virus-induced systemic inflammation adopt a novel quiescent cell state.

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TRANSIENT AND PROLONGED EPIGENETIC DISTURBANCES: EFFECT ON CELL IDENTITY AND TRANSFORMATION

Kosuke YAMAGUCHI

DNA methylation is an essential epigenetic mark that controls gene expression and genome stability. The cellular DNA methylation patterns are initially deposited by de novo DNA methyltransferases: DNMT3A and DNMT3B. Then at every ensuing round of DNA replication, the parental methylation pattern is re-established on the new DNA strands by the maintenance DNA methyltransferase, DNMT1. This enzyme itself is dependent on the activity of another protein, UHRF1, which binds hemimethylated DNA, mono-ubiquitinates histone H3, and releases the enzymatic activity of DNMT1. UHRF1 is overexpressed in cancer and may be oncogenic.
While UHRF1 is undoubtedly an activator of DNMT1, some key questions are still unanswered: does it have DNMT1-independent functions and, if yes, which one(s)? And what are the physiological consequences of UHRF1 removal?
To investigate these questions, we used the Auxin-Inducible Degron system to induce the total and synchronous depletion of endogenous UHRF1 and/or DNMT1 proteins in human cancer cells. Phenotypically, UHRF1 and/or DNMT1 depletion cells showed cell proliferation defect and senescence phenotype, and UHRF1 depletion cells showed more severe phenotype than DNMT1 depletion cells. We then performed RNA-seq, WGBS, and proteome analyses at regular time intervals, coupled with rigorous bioinformatic analysis, to uncover the molecular consequences of the treatment. This dataset shows that UHRF1 acts not only upstream of DNMT1, but also upstream of DNMT3A, which themselves counteract active demethylation by the TET enzymes.
Our molecular data reveal that UHRF1 controls more than just DNMT1, and our cellular assays show that inactivating UHRF1 can effectively drive cancer cells to senescence, which may have implications for therapy.