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MEETING REPORT

Meeting report – Building the Cell 2018

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ABSTRACT

Cell biologists from all around the world gathered in Paris on the 26 to 28 September 2018 to participate in the 3rd international meeting 'Building the Cell'. It was organized by Hélène Barelli, Arnaud Echard, Thierry Galli, Florence Niedergang, Manuel Théry and Marie Hélène Verlhac on behalf of the French Society for Cell Biology (SBCF) at the Institut Pasteur. Around 230 participants joined the meeting for stimulating talks, discussions, poster sessions, and a gala dinner on the Seine that included a music performance by the rock group 'Membrane Band'. The unifying theme of the meeting was the development of creative multidisciplinary approaches to understand cellular life at different scales in a dynamic and quantitative manner. Here, we summarize the results presented at the meeting and the emerging ideas from the different sessions.

The 3rd international meeting 'Building the Cell' (Fig. 1; Fig. 2) was divided into ten different sessions that covered a variety of topics including intracellular trafficking, cell division, cytoskeletal dynamics and cell mechanics, cancer and stem cell biology, embryonic development and tissue morphogenesis, neurobiology, and aggregates and phase transitions. A broad spectrum of modern approaches and experimental systems ranging from synthetic biology and stem cell technologies to 3D organoids and animal models was presented. The meeting highlighted some of the latest and novel findings in cell biology, often coupled to major methodological developments in quantitative microscopy and computational modelling, as well as cell and tissue micro-engineering.

Membrane, intracellular trafficking and mechanics

Thanks to the synchronized secretory retention using selective hooks (RUSH) system they developed a few years ago (Boncompain et al., 2012), Franck Perez and his team (Institut Curie, Paris, France) were able to identify specific 'hot spots' of protein secretion at focal adhesions (FAs) (Fourriere et al., 2018 preprint). The combination of the RUSH system and multispectral fluorescence live-cell imaging revealed that these hot spots emerge owing to a spatial bias in the delivery of exocytotic membranous vesicles that contain secreted proteins. Perez discussed that this bias is introduced because of two distinct cellular activities. First, because of microtubules (MTs) that grow from the cell center to the cell periphery and stochastically 'target' FAs. There, MTs pause and serve as transport routes for the targeted delivery of secretory vesicles. Second, through the Rab6–ELKS pathway, with Rab6 being systematically loaded onto post-Golgi vesicles and the ELKS complex being enriched at FAs. Interestingly, among the proteins secreted in this fashion are collagens, which are fundamental to the formation of the extracellular matrix (ECM) and promote FA formation. This suggests the existence of a hitherto unappreciated positive-feedback loop between FA assembly and localized deposition of structural proteins of the ECM.

Along these lines, Sandrine Etienne-Manneville (Institut Pasteur, Paris, France) showed some interesting results that suggest a crucial role of MTs in the mechanosensitivity of cell migration: integrin-mediated cell–substrate interactions lead to stabilization of a subset of MTs. Cytoplasmic MTs often terminate at FAs, where they accumulate post-translational marks in the form of tubulin acetylation. This process is executed by the enzyme tubulin acetyltransferase αTAT1, which resides in the vicinity of FAs (Bance et al., 2018 preprint). Acetylated stable MTs linked to FAs serve as transport routes for selective delivery of Rab6-dependent membrane vesicles to FAs, which promotes FA turnover. Experimentally interfering with the stabilizing acetylation of MTs in the vicinity of FAs leads to defects in FA growth and cell migration.

Thomas Wollert (Institut Pasteur, Paris, France) showed how selectivity in autophagy is regulated in yeast at a molecular level. By reconstituting the initiation of autophagy from purified components, he showed that the initiation of selective autophagy is intimately linked to the availability of autophagic cargo. Thus, the decision to generate a selective or a non-selective autophagosome is already made by the time of the biogenesis initiation.

Christophe Lamaze (Institut Curie, Paris, France) presented recent data on the role of caveolae mechanics in cell signaling (Torrino et al., 2018). The Lamaze research group found that, in response to mechanical stress (e.g. cell substrate stretching and osmotic cell swelling), plasma membrane caveolae undergo disassembly. Caveolae disassembly appears to be involved in processes other than membrane dynamics and homeostasis. One such process is associated with the ability of caveolin-1, a small cytoplasmic caveolae-coating protein, to modulate critical signaling pathways in mechanically challenged cells.

Combining quantitative live-cell microscopy and theoretical tools from statistical physics, Jian Liu (NIH, Bethesda, USA) presented data from his collaboration with Min Wu (Mechanobiology Institute, Singapore). He showed that the rapid changes in immune cell organization that are associated with immune responses might be wired by ultra-fast traveling waves at the plasma membrane–actin cortex interface. These waves emerge in a rhythmic manner owing to the coupling between microscopic undulations of the plasma membrane, local accumulation of the peripheral membrane proteins that are able to sense membrane curvature and to promote local actin polymerization (e.g. F-BAR domain-containing proteins) and F-actin assembly (Wu et al., 2018). Understanding how these high-speed cortical signal transduction rhythms contribute to membrane trafficking events during immune responses is one of the exciting directions for future studies by the investigators.

Nicolas Melis from the research group of Roberto Weigert (NIH, Bethesda, USA) introduced the intravital subcellular microscopy

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Journal of Cell Science (2019) 132, jcs229765. doi:10.1242/jcs.229765

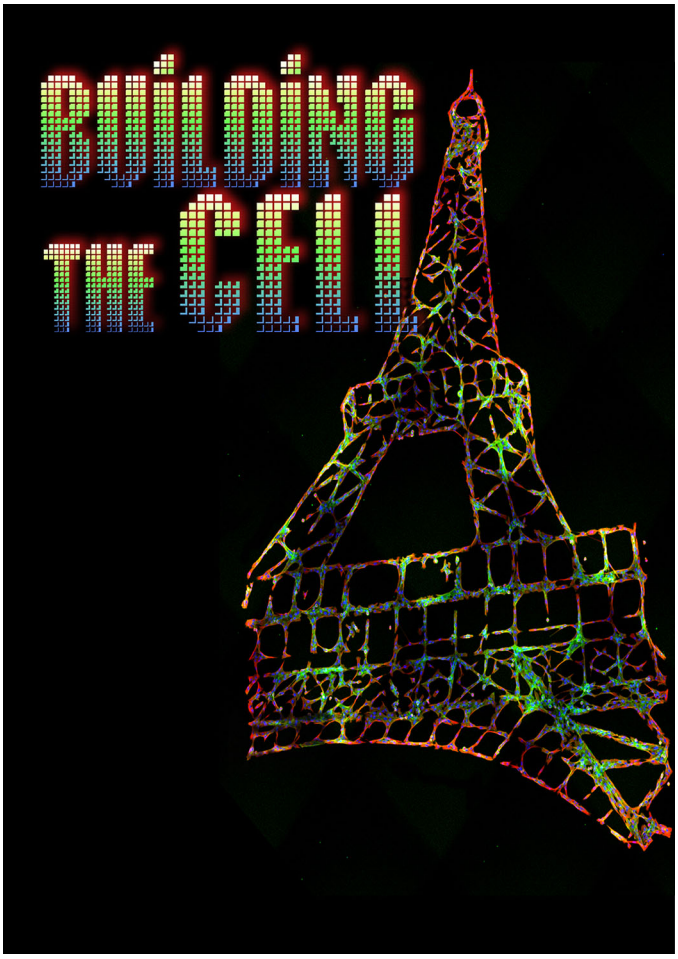

Fig. 1. Conference poster. This displays retinal pigment epithelial (RPE1) cells plated on an Eiffel Tower pattern (3 mm high), micropatterned with PRIMO (Alveole), and stained for fibronectin (red), actin (phalloidin, green) and DNA (Hoechst, blue). ©Manuel Théry.

(ISMic) technique that uses multiphoton live-cell imaging coupled to quantitative image analysis algorithms to characterize the spatiotemporal behavior of cytoplasmic membranous organelles and the actomyosin cortex during neutrophil migration in the mouse ear. His analysis revealed an unexpected pattern of myosin II distribution in migratory neutrophils *in vivo*: in addition to its widely described presence at the back of the cell, myosin IIA dynamically localizes at the cell front and lateral parts of the cell, and these accumulations seem to correlate with the presence of cytoplasmic mitochondria. These observations highlight a potential cross-talk between energy-producing mitochondria and the energy-consuming contractile actomyosin cortex.

Fig. 2. The Membrane Band performing during the gala dinner on the Seine. ©Membrane band.

Cell division and cancer

Cell division is controlled by multiple factors, and its misregulation is often associated with cancer formation. Thus, understanding the cellular and molecular mechanisms behind cell division is of fundamental biomedical importance. During cell division, the spindle apparatus, which comprises MTs and centrosomes, ensures that chromosomes segregate evenly. Using computational simulations and experiments, Renata Basto (Institut Curie, Paris, France) revealed how numerical abnormalities in chromosomes and centrosomes could affect centrosome clustering and chromosome partitioning in polyploid cells. Susana Godinho (Barts Cancer Institute, London, UK) discovered a new mechanism through which numerical deviations in centrosome numbers can affect complex cell behaviors. She showed that centrosome amplification in a subset of cells can induce cancer-like phenotypes in neighboring cells with normal centrosome numbers. This non-cell-autonomous mechanism is based on the increased secretion of interleukin 8 (IL8) in cells with supernumerary centrosomes. The secreted IL8 can switch on a paracrine signaling axis that drives invasive migration of neighboring cells with normal centrosome numbers, both in 3D mammary organoids and zebrafish models (Arnandis et al., 2018).

Centrosomes control the mitotic spindle at the poles, whereas kinetochores, connecting spindle microtubules to chromosomes, operate at the equator. How are these two types of activities coordinated to achieve proper chromosome segregation? What are the major forces governing this process? Iva Tolić (Ruđer Bošković Institute, Zagreb, Croatia) presented a detailed analysis of mitotic spindle architecture near kinetochores. By using expansion microscopy, a type of super-resolution imaging, and optogenetic tools, her team discovered that ‘bridging fibers’ connect sister kinetochore fibers. These bridging fibers maintain proper tension at the kinetochores. In addition, she presented how, as well as to ‘pulling’ and ‘pushing’ forces, rotational forces (torques) can emerge in the spindle; this was evidenced by the chirality of the spindle that they observed using the newly developed analytical tools (Novak et al., 2018).

Septins are less-well studied cytoskeletal proteins that play a major role in cell division and cytokinesis. Through *in vitro* purification approaches, microfabricated grooves and electron cryotomography, Aurélie Bertin (Institut Curie, Paris, France) showed that septins are able to discriminate between distinct types of micron-scale substrate curvature. The proteins follow positive substrate curvature, but align perpendicularly to the substrate when its curvature is negative. These local curvature-sensitive supramolecular rearrangements of septins appear to remodel membranes, thus illuminating the structural mechanisms through which septins can contribute to membrane deformations accompanying cleavage furrow formation and ingression in cells.

Manuel Mendoza (Institute of Genetics and Molecular & Cellular Biology, Illkirch, France) demonstrated that, in addition to cytoskeletal proteins, chromatin and DNA-associated processes can also regulate cell division. Indeed, how are cells aware that DNA replication is complete before cell division? He discussed data from his research group showing that during unperturbed growth of

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budding yeast, DNA replication finishes in anaphase. This step is required to resolve chromatin bridges and to complete cytokinesis, thus enabling faithful and complete segregation of chromosomes (Ivanova et al., 2018 preprint).

Organoids and stem cells

This session focused on the behavior of multicellular organoids and introduced novel tools such as organ-on-chip devices that aim to mimick mammalian tissues and organs. Delphine Delacour (Institut Jacques Monod, Paris, France) presented how the cell–cell junction protein epithelial cell adhesion molecule (EpCAM) can regulate the biomechanics of intestinal epithelium (Salomon et al., 2017). EpCAM loss is responsible for a rare disease – congenital tufting enteropathy (CTE). Delacour discussed how her team designed 3D substrates to mimic specific aspects of intestinal tissue geometry and to model the disease in a dish. Unlike what is seen in 2D epithelial monolayers, EpCAM-deficient epithelia on the fabricated 3D substrates displayed a phenotype similar to that found in CTE. The role of EpCAM in the control of CTE is based on its surprising ability to regulate the actomyosin cytoskeleton independently from the role of the protein in cell–cell junction formation. Combining *in vivo* imaging in mice and computational modeling, Daniela Vignjevic (Institut Curie, Paris, France) showed that active cell crawling is essential for homeostasis of the gut epithelium. Her group found that a steady cell movement – observed during homeostatic processes that are associated with continuous cell renewal – within the epithelium is independent from cell division, long thought to be the major contributor. Instead, she discussed that gut epithelial cells migrate in a manner dependent on the actin-regulating Arp2/3 complex. Her research group is currently using a biomimetic 'gut-on-chip' platform fabricated out of natural collagen to recapitulate crypts and villi to probe deeper into the details of their discovery. To study the molecular and cellular mechanisms behind urinary tract infections in humans, Jennifer Rohn (University College London, UK) highlighted the development of a human urothelial organoid model. Using this system, her research group is modeling and analyzing the process of urothelial invasion and colonization by the bacterial pathogen *Enterococcus faecalis* (Horsley et al., 2018).

How are signaling pathways and mechanical cues coupled within a tissue to maintain its normal structure and function? Kevin Chalut (University of Cambridge, UK) revealed a feedback loop between ECM mechanics and stem cell signaling in the context of neural stem cell plasticity in the rat brain. He discussed that aging-associated stiffening of the ECM in the brain tissue is sensed by central nervous system progenitor cells. This leads to a loss of function in the progenitor cell population, as demonstrated by the fact that the cells exit the cell cycle and are unable to differentiate. Disrupting mechanosensing factors allows the progenitor cell population to maintain its undifferentiated state regardless of ECM stiffness, paving a way for means to rejuvenate adult brain tissue.

Cell biology and embryonic development

Jean-Léon Maitre (Institut Curie, Paris, France) utilizes live-cell microscopy and quantitative analyses of forces responsible for deformation and motion of cells during mouse preimplantation development. Using this approach, he presented recently discovered novel biomechanical mechanisms that drive blastocoel formation in the blastocyst. Magdalena Zernicka-Goetz (University of Cambridge, UK) gave the EMBO keynote lecture, during which she presented fascinating new results from her research on the reconstitution of early post-implantation stage human and mouse embryos *in vitro* (Sozen et al., 2018). She highlighted the importance of self-assembly and self-organization in the process of construction of these synthetic embryos even in the absence of environmental cues. Using early *Drosophila* embryos, biophysical tools and quantitative microscopy of intercellular junctions in the embryonic epithelium, Pierre-François Lenne (Developmental Biology Institute of Marseille, France) showed how force balance and tension at the junctional interface can be inferred in a non-invasive manner. His team found that the vinculin-to-E-cadherin ratio at cell–cell contacts correlates with tension and could serve as a reliable readout of mechanical effects of myosin II on intercellular junctions. Analytical tools such as those presented by Pierre-François will not only provide new fundamental insights into the cell biology and biophysics of early embryonic development, but also benefit the entire community of scientists studying epithelial cell biology.

During the early stages of embryogenesis, cell crowding due to proliferation and extensive tissue remodeling creates confining environments within the developing embryo. Alexis Lomakin (King's College London, UK) addressed the question of how epithelial cells sense and adapt to spatial confinement within tissues. To answer this question, he collaborated with biophysicists and bioengineers from the research groups of Matthieu Piel (Institut Curie, Paris, France) and Daniel Müller (ETH Zürich, Switzerland) to experimentally constrain live cells. By doing so, he and his colleagues found that cells can estimate the degree of their spatial confinement thanks to the nucleus, which serves as a built-in ruler of environmental cell confinement. The ruler function of the nucleus is based on the limited nuclear envelope area that can be stretched in response to nuclear compression. This in turn engages nuclear membrane stretch-sensitive proteins and a signaling downstream of their activity to ultimately increase cortical actomyosin contractility and thus mechanical resistance to excessive cell confinement. Romain Levayer (Institut Pasteur, Paris, France) presented his recent work on the role of mechanical cues in cell fate choices. Romain developed a new live fluorescence sensor of an extracellular signal-regulated protein kinase (ERK; known as Rolled in flies) to monitor how the activity of this critical signaling molecule correlates with distinct spatiomechanical inputs in the *Drosophila* pupal notum. Using functional genetics coupled to biophysical manipulations and quantitative microscopy, he showed that local tissue stretching transiently upregulates the activity ERK, which correlates well with cell survival. Whereas local tissue compaction or compression decreases the levels of active ERK and serves as a potent inducer of cell death, cells in which ERK activity levels were artificially increased gain competitive advantage as they overproliferate, increasing tissue density, and compress neighboring cells to death. This mechanical competition can be a generic mechanism for tissue patterning.

Cellular neurobiology

Lukas Kapitein (Utrecht University, Netherlands) presented how he utilizes state-of-the-art microscopy methodologies (nanometric tracking and optogenetics) to reveal the logistics of protein transport and the organization of MT 'highways' in axons and dendrites. By using motor proteins as a probe for MT polarity and organization, his research group found that different MT subsets recruit distinct motor proteins and have antiparallel orientations in dendrites, but not in axons. This explains why certain kinesins only move into axons, whereas others can enter both axons and dendrites (Tas et al., 2017). In addition, Lukas introduced a new assay to induce and follow selective autophagy (Janssen et al., 2018). Anne Bertolotti (MRC Laboratory of Molecular Biology, Cambridge, UK) described the identification of a new small molecule called Raphin1 that is capable of boosting protein quality control through

selective inhibition of a phosphatase (Krzyzosiak et al., 2018), preventing protein misfolding. This work opens possibilities for therapeutic treatments against neurodegenerative diseases.

Aggregates and phase transition

Similar to oil droplets fusing together once they are dissolved in water, components of the protein biosynthesis machinery in the cytosol or RNA-processing factories in the nucleoplasm often undergo liquid unmixing and form droplet-like assemblies for protein or RNA compartmentalization, storage or facilitation of biochemical reaction rates. Curiously, these droplets are often only observed in interphase and undergo dissolution when cells divide.

Arpan Rai, a postdoctoral fellow from the research group of Lucas Pelkmans (University of Zürich, Switzerland), presented their recent work (Rai et al., 2018) identifying dual-specificity tyrosine-phosphorylation-regulated kinase 3 (DYRK3) as a dissolvase for several types of cytoplasmic and nuclear droplet-like condensates in mitosis. Compared to the substrates of DYRK3 that are confined either to the cytoplasm or to the nucleus, DYRK3 itself localizes to both of the compartments in interphase cells. However, upon nuclear envelope breakdown, the cytoplasmic and nuclear substrates get diluted relative to the concentration of DYRK3 as a consequence of nucleo-cytoplasmic mixing. Arpan proposed a model in which the dilution of phase-separating proteins, together with the kinase activity of DYRK3, induces dissolution of the condensates. What is the biological significance of dissolving the liquid unmixed structures during mitosis? It turned out that the presence of droplet-like assemblies in mitotic cytoplasm leads to the formation of ectopic nucleation centers for phase separation of critical molecular components of the mitotic spindle. This strongly affects the organization of the mitotic spindle and results in mitotic abnormalities inducing mitotic arrest. To further understand the biological role of phase separation, Clifford Brangwynne (Princeton University, USA) presented optogenetic tools that are meant to induce the assembly of membrane-less condensates of RNAs and RNA-binding proteins (‘optodroplets’) in a controlled fashion (Bracha et al., 2018; Shin et al., 2018). These tools can be used to study the role of nuclear liquid demixing in gene expression. Indeed, he showed that nuclear condensates are not randomly dispersed within the nucleus; instead, they tend to localize to heterochromatin domains of low density and exclude chromatin as they grow, therefore deforming chromatin around themselves. Do these localized chromatin deformations regulate transcription? Answering this question is an exciting direction for future research. In budding yeast, phase separation may occur during metaphase to ensure proper cell division. Yves Barral (ETH Zürich, Switzerland) focused his presentation on the *Saccharomyces cerevisiae* protein KAR9, related to the protein adenomatous polyposis coli (APC) in humans, which localizes at the plus end of cytoplasmic MTs in metaphase cells. Together with the research group of Michel Steinmetz (PSI, Villingen, Switzerland), he dissected how KAR9 interacts with the protein Bim1 (the homologue of mammalian end-binding protein 1; EB1) (Manatschal et al., 2016). His current data indicate that Kar9 and Bim1 phase-separate *in vitro*. It will be interesting to determine whether these proteins also form droplets in cells and if they do, how and whether this contributes to their function in promoting the segregation of the old spindle pole body to the bud during yeast cell division.

Concluding remarks

Looking over the landscape of the cell biology presented at this meeting, it was multidimensional, extremely interdisciplinary and collaborative, diverse and inclusive. We left the meeting galvanized by the exciting ideas, equipped with novel tools, and supported by newly established collaborations; this is all thanks to the organizers and participants of the ‘Building the Cell’ meeting 2018, making it a great success!

Acknowledgements

We apologize to our colleagues whose work could not be discussed here due to space limitations.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was supported by the King’s Prize Fellowship & London Law Trust Medal Fellowship (grant MGS9403 ‘Mechanobiology of cell fate switching’) and by a travel fellowship from Boehringer Ingelheim Stiftung to attend the ‘Building the Cell’ Meeting – 2018 to A.L.

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