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MEETING REPORT

Meeting report – Dynamic Cell III

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ABSTRACT

Dynamic Cell III, a meeting jointly organized by the British Society of Cell Biology (BSCB) and the Biochemical Society, took place at the Manchester Conference Centre, Manchester, UK in March 2018. It brought together a diverse group of scientists from around the world, all with a shared interest in understanding how dynamic functions of the cell are fulfilled. A particular focus was the regulation of the cytoskeleton: in cell division, cell migration and cell-cell interactions. Moreover, a key theme that ran through all presented work was the development of new and exciting technologies to study dynamic cell behaviour.

Dynamic Cell III was the third in a series of joint meetings between The Biochemical Society and BSCB, designed to showcase the very latest cell biological research, exemplifying the use of dynamic methods and technologies. The organizing committee (Jeremy Carlton, Anne Straube, Thomas Surrey, Guillaume Charras, Sarah Woolner and Theresa Ward) drew from both societies and used their diverse scientific backgrounds to build a programme that covered a range of disciplines and biological scales: from structural biology through to organoid cultures and embryology. A unifying theme was a shared fascination with the workings of the cell and a belief that the only way to understand their regulation is to investigate cells using the most dynamic approaches.

Following an excellent student and postdoc pre-meeting symposium, Tony Hyman (MPI-CBG, Dresden, Germany) kicked off the full meeting with a typically inspiring keynote lecture on phase separation. In order for cells to organize and concentrate their biochemical reactions, compartmentalization is crucial. This can be achieved by membrane-bound organelles, but non-membrane-bound compartments, for instance P-granules and stress granules, provide a highly dynamic alternative (Wheeler and Hyman, 2018). Many of these compartments are liquid-like – fusing and dripping like liquids – and form by phase separation, like a droplet of oil forms in water. Tony's lab has been investigating whether phase separation can explain the pathological aggregates of prion-like proteins.

Imaging and probing cell function

Advances in imaging technologies have been instrumental in elucidating the wide-ranging behaviour and function of the cell. Andy Oates (EPFL, Switzerland) shared his work on the regulation of vertebrate segmentation focusing on the origin of 'waves' of synchronized gene expression (Liao and Oates, 2017). Looking at oscillations in gene expression, he observed that single cells isolated *in vitro* slow their oscillations and differentiate, suggesting that the key processes underlying the tissue level 'wave' of gene expression do not depend on long-range signals or cell-to-cell contact. This implies instead that a cell-autonomous timer is involved in regulating the ordered segmentation of vertebrae. Thomas Surrey (The Francis Crick Institute, London, UK) described the use of a motor and microtubule self-organisation assay to identify critical parameters that control the self-organisation process of a microtubule or motor network. These concepts not only provide insight into how the shape of the mitotic spindle is defined but also how distinct spindle defects result from particular molecular perturbations.

The discovery and development of new imaging techniques is fundamental to our ability to answer the outstanding questions in biology. Michelle Peckham (University of Leeds, UK) introduced affimer technology: artificial non-antibody binding proteins that can be selected against a range of protein targets. Michelle described four actin-binding affimers, which work in both fixed and live cells (Lopata et al., 2018) and affimers for tubulin, which can bind to specific microtubules or, potentially, specific post-translational modifications. New imaging methods to study chromatin compaction were presented by Abder Kaidi (University of Bristol, UK). Although the compaction of DNA before and during cell division has been closely studied, relatively little is known about its decompaction after cell division. Using measures of chromatin compaction over time to observe the behaviour of DNA at the end of cell division, Abder observed that the depletion of actin during nucleus reformation prevented nuclear expansion (Baarlink et al., 2017).

In a further example of how cutting edge imaging can provide important mechanistic insight, Martin Schwartz (University of Manchester, UK, and Yale University, New Haven, USA) presented his work on the transmission of force through focal adhesions. With the use of a FRET sensor to measure forces between proteins and speckle microscopy to observe the flow of actin along focal adhesions, he described a model for force transfer where the requirement for actin flow is dependent on cell location. Yohei Yamauchi (University of Bristol, UK) demonstrated the power of increasing resolution in live imaging for deciphering the endocytic uptake of viruses into living cells. Using a combination of atomic force microscopy and confocal microscopy (BIXAM, Olympus), it was possible to directly observe the uptake of influenza viruses through the plasma membrane of living cells, an event that previously could only be seen in fixed electron micrographs. Using this new approach, he found that the majority of viruses are taken up through a clathrin-dependent pathway or through pinocytosis, but novel methods of virus uptake were also observed and are now being explored further.

Cytoskeletal dynamics

The cytoskeleton is a key driver and regulator of dynamic cell functions, and was explored during numerous sessions of the meeting. One particular focus was motor proteins, which use the hydrolysis of ATP to move along cytoskeletal filaments and are essential for many dynamic functions, including cargo transport,

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Journal of Cell Science (2018) 131, jcs222927. doi:10.1242/jcs.222927

organelle positioning, cell division and cell motility. Anne Straube (University of Warwick, UK) described her work investigating the activation of the kinesin KIF1C, whose transport to the plus-end of microtubules is required to stabilize the cell rear for motility (Efimova et al., 2014). Cross-linking mass spectrometry suggested that KIF1C is held in an auto-inhibitory configuration, in which the tail domain interacts with the motor domain. Anne and her team found that the tyrosine phosphatase PTPN21 activates KIF1C by relieving this inhibition and converting KIF1C into a processive motor. Isabel Palacios (Queen Mary University London, UK) discussed the role of cytoskeletons in the motion of cytoplasmic components. Using the Drosophila oocyte as a model system, and applying differential dynamic microscopy as a novel method for motion characterization, she showed that cytoplasmic motility constitutes a combination of directed motion and random diffusion. Importantly, this work sheds new light on the dynamic interplay between ATP-dependent forces and cytoplasmic mechanics in the regulation of intracellular motility (Drechsler et al., 2017).

Motor proteins are often hijacked by pathogens, and the study of bacterial infection has made a major contribution to our understanding of cytoskeletal dynamics. Serge Mostowy (Imperial College London, UK) used infection of cells by the bacterium *Shigella* to gain insight into the role of septins, which have been coined the fourth member of the cytoskeleton. Serge showed how septins recognize micrometre-scale membrane curvature and bind specifically to dividing bacteria inside the cell forming a cage-like structure, which targets them for destruction by autophagy (Mazon-Moya et al., 2017). Thus, the study of septin-mediated cellular immunity may point to a novel way to treat infection. The main means through which a host organism protects itself against infection is through immune cells that engulf microbes through phagocytosis. The actin cytoskeleton, which is regulated by the Rho and Ras GTPases, is essential for the formation and function of the phagocytic cup and Jason King (University of Sheffield, UK) presented the identification of a novel regulator RGBarG, describing how it spatially coordinates Ras and Rac GTPases across the phagocytic cup. The regulation of cytoskeletal dynamics is also essential for antigen recognition by T-cells, a key step in the adaptive immune response. Huw Colin-York (University of Oxford, UK) described how actin dynamics normalize the force induced by antigens in an antigen specific manner. This provides a system by which the length and time-scale of T receptor signalling is dictated by feedback between antigen kinetics and cytoskeletal dynamics.

The Biochemical Society GlaxoSmithKline Award 2018, given in recognition of pioneering research that leads to new advances in medical science, was presented to Anne Bertolotti (MRC-LMB, Cambridge, UK). In her presentation, Anne highlighted the selective inhibition of phosphatases as an attractive therapeutic approach for the treatment of neurodegenerative diseases (Tsaytler et al., 2011). A hallmark of these diseases is the accumulation of protein aggregates due to failing quality control mechanisms associated with age. By manipulating the innate stress response using selective phosphatase inhibitors, Anne showed that it is possible to decrease protein synthesis and thus increase protein repair in the context of failing, over-loaded, quality control mechanisms. This prevents the catastrophic accumulation of misfolded proteins that leads to neurodegeneration.

Cytoskeletal regulation of cell division

Cell division is an inherently dynamic process and work presented at Dynamic Cell III demonstrated the use of cutting-edge technologies to make crucial new discoveries about this fundamental process. Isabelle Vernos (CRG-ICREA, Barcelona, Spain) presented a mass spectroscopy approach using *Xenopus* egg extracts to identify new proteins involved in the self-organisation of microtubules into spindles. In addition to the identification of new spindle proteins, this proteomic profiling approach also gave an indication of protein dynamics and suggested that different groups of proteins are recruited to the spindle in a time-dependent manner (Rosas-Salvans et al., 2018). Lori Borgal (University of Exeter, UK) described her efforts in investigating the role of abnormal spindle-like microcephaly-associated protein (ASPM), a microtubule-associated protein that is frequently truncated in human microcephaly (Bond et al., 2002). Lori investigated how mutating the protein phosphatase 2a (PP2A) binding motif in the *Drosophila* homologue of ASPM, Asp, caused unfocused spindle poles in the optic lobe stem cells – a phenotype that appears to be specific to neural stem cells and was associated with significant developmental delay.

While the role of microtubules and microtubule-associated proteins is clearly central to mitotic and meiotic spindles, novel roles for actin are also being uncovered. Binyam Mogessie (University of Bristol, UK) described his observations of actin filaments in the meiotic spindle across a range of mammalian eggs, including those of humans. Disruption of this actin structure in either meiosis I or meiosis II interferes with the function of K-fibres, the microtubules that usually bind the kinetochore and mediate proper chromosome alignment and segregation. The function of this meiotic spindle actin is therefore required to prevent chromosome segregation errors that lead to aneuploidy in eggs, which in turn, is associated with decreased fertility and developmental disorders (Mogessie and Schuh, 2017). Natalia Wesolowska (EMBL, Heidelberg, Germany) presented another novel role for actin in driving nuclear envelope breakdown in starfish oocytes. In somatic cells, microtubules facilitate the breakdown of the nuclear envelope; however, starfish oocytes are much larger and microtubules are too short to bridge their 80-µm-diameter nucleus. Instead, Natalia described how nuclear envelope breakdown in these oocytes is mediated by an F-actin ‘shell’ that develops on the inner surface of the nuclear envelope and drives its fragmentation through a microtubule-independent process (Mori et al., 2014).

Dividing cells normally contain two centrosomes, one at each pole of the mitotic spindle, and maintenance of this number is crucial for spindle structure and accurate chromosome segregation. Susana Godinho (Queen Mary University London, UK) presented her work on how transformed cells are able to tolerate centrosome amplification by clustering them together. However, for this to occur E-cadherin needs to be lacking, since loss of E-cadherin increases cortical contractility, allowing more efficient clustering. In breast cancer cell lines, reduced E-cadherin is correlated with both increased centrosome amplification and clustering, indicating that this mechanism may be relevant in cancer progression (Rhys et al., 2018).

Mitotic spindles have an inherent ability to align with the long axis of the cell, a phenomenon known as Hertwig’s rule. Bénédicte Sanson (University of Cambridge, UK) presented her group’s new findings indicating that there is a region within the *Drosophila* embryonic epidermis in which cell division does not follow the long-axis rule. They showed that the presence of an actomyosin cable at the parasegmental boundary provides an area of localized tension and acts to reorient cell divisions perpendicularly to the boundary, against their long axis (Scarpa et al., 2018 preprint). Dan Bergstralh (University of Rochester, NY, USA) described another example of Hertwig’s rule being broken in the maturing *Drosophila* egg chamber. He reported that neither the position of tricellular

Journal of Cell Science (2018) 131, jcs222927. doi:10.1242/jcs.222927

junctions nor interphase shape predicts cell division orientation in this context, instead divisions align along the elongating axis of the egg chamber. Dan suggested that this implies a tension-dependent mechanism of aligning cell division that is independent of cell shape.

Andrew McAinsh (University of Warwick, UK) was awarded the 2018 BSCB Hooke Medal, which recognizes emerging leaders in cell biology, for his work on the mitotic spindle. During his award lecture, Andrew described the role of the kinetochore as a molecular machine that ensures error-free segregation of the chromosomes (Auckland and McAinsh, 2015). Over the past decade or so, Andrew's lab has played a pivotal role in increasing our understanding of this fundamental and yet complex machine. Andrew described the exciting new imaging technologies being developed in his lab to determine the relative positions of kinetochore proteins and how kinetochore conformation changes when microtubules attach. Pulling this wealth of data together provides a much clearer understanding of the kinetochore and sheds important light on how the interactions of different modules of the kinetochore can give rise to its emergent properties.

Cell migration and the extracellular matrix

The use of 3D systems for studying dynamic cell behaviour was an emerging theme of the meeting, and was especially evident in the session on cell migration and the extracellular matrix (ECM). For cancer biology, it is clear that the classical 2D models utilized for investigating cell migration do not adequately represent the complex tumour microenvironment. David Mason (University of Liverpool, UK) presented a 4D imaging approach using tumour spheroids in ECM to track individual and bulk migration in a physiologically relevant context (Marcello et al., 2017). David also described a novel approach for post-acquisition analysis of tracking data in order to elucidate unique characteristics of cells that invade the surrounding ECM matrix, or to delineate the mechanism of action for anti-cancer drugs. Peter Friedl (University of Nijmegen, The Netherlands, and MD Anderson Cancer Center, Houston, USA) discussed how cancer cells migrate in a collective manner (Khalil et al., 2017), unlike the single-cell migration mode commonly observed *in vitro*. It is widely thought that proteolytic degradation of the ECM is required for cancer cell invasion into new tissue. However, Peter showed that invasive cancer cells follow patterns of, for instance, myofibres and nerves by adapting to the pattern of the pre-existing ECM mesh. Therefore, tissue destruction during invasion is an inevitable consequence of, but not a prerequisite for, cancer cell migration.

The leading edge of a cell during migration has previously been considered the main instigator of movement; however, Brian Stramer (King's College London, UK) argued that actin polarity and actin flow are crucial for determining the direction of travel. Cell-wide actin flow is globally coordinated, persistent, and concurrent with cell motion. The importance of actin and myosin flows was further emphasized by Raphaël Voituriez (Université Pierre et Marie Curie, Paris, France), who showed that they are a conserved feature of eukaryotic cells that underpins a plethora of migration modes (Moreau et al., 2018). In collective cell migration, the establishment of front-rear polarity is known to be vital but, while much is understood about cell behaviour at the leading edge, much less is known with regard to the cells at the back of the migrating group. Adam Shellard (University College London, UK) described how a contractile supracellular actomyosin ring at the rear of migrating neural crest cell groups helps drive their collective chemotaxis.

Focal adhesions (FAs) provide the physical link between ECM and the actin cytoskeleton. Lorna Young (Dartmouth College, Hanover, USA) spoke about her recent efforts in dissecting the morphology of FAs in cell attachment and migration. Using live-cell imaging, Lorna observed that the assembly of mature FAs during cell spreading occurs through splitting of large FA plaques, with the resulting FA units varying in length, but having a remarkably constant width of 0.3 µm. This morphology is observed in different cell types on different matrices, suggesting the existence of a 'molecular ruler' that defines the width of a FA unit.

Cell-cell interactions

To coordinate behaviour in dynamic tissue environments, cells need to be able to communicate with each other and respond appropriately. Alpha Yap (University of Queensland, Brisbane, Australia), whose talk was sponsored by The Royal Microscopical Society, presented his lab's recent work identifying a novel protective mechanotransduction mechanism operating at epithelial junctions. In this pathway, the application of external stress transmitted through E-cadherin stabilizes the mechanosensory protein myosin VI, ultimately leading to the downstream activation of RhoA. The resultant reorganization of actomyosin strengthens the adherens junction and serves as a protective mechanism to maintain epithelial homeostasis (Acharya et al., 2018 preprint). Staying with Rho, Ann Miller (University of Michigan, Ann Arbor, USA) highlighted in her talk a protective role for Rho 'flares' (Reyes et al., 2014), which locally reinforce the tight junction barrier in response to mechanical stress. Using a novel zinc-based ultrasensitive microscopic barrier assay, they were able to observe local breaches in the barrier prior to bursts of RhoA activity and the resultant repair of the barrier.

The perturbation of normal cell-cell interaction by cancer was discussed in a number of talks. Chiara Francavilla (University of Manchester, UK) presented data from a functional proteomic approach to investigate the role of aberrant FGFR signalling in breast cancer progression. By stimulating distinct breast cancer cell lines with FGFs at different time points, Chiara was able to identify FGFR-signalling networks by mass spectrometry, proteomics, bioinformatics and experimental validation, which, taken together, give rise to a novel endocytic signature in breast cancer cells (Francavilla et al., 2016). Simon Brayford (King's College London, UK) highlighted recent work on contact inhibition of locomotion by using a new *in vitro* model of cell sorting where HT1080 fibrosarcoma cells and HaCaT epithelial cells, which repel each other, are plated side-by-side. Knocking down the Ephrin receptor EphB2 in the fibrosarcoma cells, or inhibiting MAPK signaling, led to a failure of cell sorting, with cells no longer being repelled by the approaching epithelial cells. Megan Moruzzi (University of Manchester, UK) presented her *Xenopus* model of early carcinoma, in which clusters of oncogene-expressing cells are created within normal *in vivo* tissue. She observed changes in cell division in wild-type cells surrounding K-Ras^{V12}- and MYC-expressing clusters, which appeared to be due to alterations in the mechanical and chemical tissue environments, respectively.

Meritxell Huch (University of Cambridge, UK) closed the conference with her BSCB 'Women in cell biology' lecture, in which she described the human liver disease model she has recently developed and her progress in translating this into potential novel treatments. Meritxell described how her lab generates human liver donor organoids, which can be expanded long term, preserving genetic stability and differentiation status. Furthermore, liver tumouroids, which maintain the histological architecture of their tumour subtype, can be successfully generated with the potential to be used for biomarker identification and drug screening for novel treatments (Broutier et al., 2017).

Concluding remarks

In summary, this meeting showcased a wealth of varied research within the field of dynamic cell biology, with scales ranging from the molecular to the whole organism. What united all the presented work was a shared awareness that biological processes can only be fully understood by studying them dynamically. Moreover, the meeting brought together an excellent mix of early-career researchers and more-experienced cell biologists, fostering a collaborative and supportive environment; we very much look forward to Dynamic Cell IV.

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