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MEETING REPORT

Meeting report – Intercellular interactions in context: towards a mechanistic understanding of cells in organs

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ABSTRACT

The Company of Biologists held the workshop 'Intercellular interactions in context: towards a mechanistic understanding of cells in organs' at historic Wiston House in West Sussex, UK, 5–8 February 2017. The meeting brought together around 30 scientists from disparate backgrounds – yet with a common interest of how tissue morphogenesis occurs and its dysregulation leads to pathologies – to intensively discuss their latest research, the current state of the field, as well as any challenges for the future. This report summarises the concepts and challenges that arose as key questions for the fields of cell, cancer and developmental biology. By design of the organizers – Andrew Ewald (John Hopkins University, MA), John Wallingford (University of Texas at Austin, TX) and Peter Friedl (Radboud University, Nijmegen, The Netherlands) – the attendee makeup was cross-sectional: both in terms of career stage and scientific background. This intermingling was mirrored in the workshop format; all participants – irrespective of career stage – were given equal speaking and question time, and all early-career researchers also chaired a session, which promoted an atmosphere for discussions that were open, egalitarian and supportive. This was particularly evident in the scheduled 'out-of-the-box' sessions, which provided an avenue for participants to raise ideas and concepts or to discuss specific problems they wanted feedback or clarification on. In the following, rather than act as court reporters and convey chronological accounting of presentations, we present the questions that arose from the workshop and should be posed to the field at large, by discussing the presentations as they relate to these concepts.

Things in motion catch the eye sooner – development, cancer and migration

Recent advances in microscopy and culturing techniques were showcased throughout the workshop, and the talks addressing cellular and even subcellular migration were no exception. These presentations highlighted recent discoveries with regard to the pathways that regulate cytoskeletal dynamics, the mechanisms of collective cell migration and collective cell invasion, as well as the molecular and behavioural differences between cells in culture and cells *in vivo*. The discussions surrounding these presentations focused on three main questions.

First, how well do our *in vitro* models of morphogenesis recapitulate *in vivo* events? This question seemed to pervade nearly every session of the workshop, and was addressed by a number of approaches. Building on previous studies (Tabler et al., 2013), Karen Liu (King's College London, UK) highlighted the essential role the environmental context plays *in vivo* by showing different populations of neural crest cells (NCCs) behave differently during migration. Tobias Zech (University of Liverpool, UK) presented a proteomics approach that identified differential protein interactions between 2D and 3D cultures, and provided evidence that 3D-specific adhesion sites are required for cell migration. These and several additional talks argued that *in vivo* mechanisms do not necessarily reflect the models generated by 2D approaches.

Second, what factors dictate cell invasiveness? Andrew Ewald showed that multi-clonal metastases arise from collective cell migration and collective invasion, and that invasive cells continue to express basal markers (Cheung et al., 2016). Keeping with this idea, Nilgun Tasdemir (University of Pittsburgh, PA) presented regulators of the actin cytoskeleton that localize to the basal domain of invasive cells, which maintains cell adherence to the extracellular matrix. Erik Sahai (The Francis Crick Institute, London, UK) demonstrated that fibroblasts can be recruited as pioneer cells during collective invasion and argued that these fibroblasts act like "a man with a machete" to carve out the path of invasion for the lagging cancerous cells (Labernadie et al., 2017). In addition, Chris Hanley (University of Southampton, UK) highlighted the role of TGFβ signalling in how cancer-associated fibroblasts (CAFs) influence tumour cell motility (Mellone et al., 2016). Irene Ylivinkka (University of Helsinki, Finland) then presented evidence that retrograde signals from lagging to leading cells promotes collective invasion (Ylivinkka et al., 2017). Keeping with this theme, Johanna Ivaska (University of Turku, Finland) demonstrated that hyperactive integrins cause an increase in filopodia and overall invasiveness, suggesting that there are mechanisms in place to keep cellular invasiveness in check (Lilja et al., 2017).

Last, can we rigorously define and classify different types of cell movement? Laura Machesky (CRUK Beatson Institute, Glasgow, UK) presented elegant work showing that small GTPases are key regulators of actin dynamics during melanocyte migration, and that actomyosin-based movement of single cells on a stationary substrate defines classic migration (Woodham et al., 2017). However, classifying other types of cell movement, either at the tissue level or at an intermediate level, would need to account for additional factors, such as the substrate being static or dynamic itself. Further complications in categorizing modes of cell motility arise at tissue level, when cells are moving along multiple axes. Needless to say, these exciting discussions emphasized that the mechanisms by which cells generate movement remain incompletely understood.

Things are shaping up – morphogens, morphogenesis and polarity

One challenge facing the field of organogenesis is to understand how the embryo can use a limited 'vocabulary' of signalling molecules to generate the myriad of cell types and cell shapes that

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comprise functional organs. The gene regulatory networks that direct large-scale patterning events, such as anterior–posterior patterning in the blastoderm embryo or proximal–distal patterning in a limb, have been characterized in great detail. The extent to which organ precursors are 'hard-wired' was debated during several discussions and the role of tissue architecture was questioned in driving cell fate specification. By using hair follicles as a model, Danelle Devenport (Princeton University, NJ) showed that, downstream of cell rearrangements are reminiscent of convergent extension, asymmetric cell morphologies can dictate cell fate decisions. Darren Gilmour (EMBL, Heidelberg, Germany) paralleled these observations by asking how 'stigmergy', i.e. indirect communication of cells with the environment, could drive differentiation. Impressively, his data showed that morphological changes in a tissue can concentrate growth factors to a single cell within a population and, in turn, promote cell fate decisions (Durdu et al., 2014). Similarly, by using the pancreas to model organogenesis (Larsen and Grapin-Botton, 2017), Anne Grapin-Botton (DanStem, University of Copenhagen, Denmark) argued that environmental inputs, specifically fluid flow in the lumen, shape the pancreatic branching network.

In a more simplified system, Marta Shahbazi Alonso (University of Cambridge, UK) presented work on the connections between pluripotency and epithelial tissue formation. There is also diversification within the signal-responding cells and Michael Way (The Francis Crick Institute, London, UK) demonstrated that different isoforms of essential actin cytoskeleton regulators, such as the Arp2/3 complex, have unique roles during myogenesis (Abella et al., 2016).

Although inductive cues are essential for organ development, several presentations focused on the role of repulsion during development. Elke Ober (DanStem, University of Copenhagen, Denmark) proposed a new mechanism of left–right asymmetry, in which repulsive cues between the endoderm and mesoderm position the developing liver (Cayuso et al., 2016), and Aaron Johnson (University of Colorado, Denver, CO) showed that multiple repulsive signals also direct myofibre morphogenesis (Williams et al., 2015). At the molecular level, Dan Fletcher (University of California, Berkeley, CA) presented evidence that protein exclusion at membrane interfaces can play an essential role in signalling and fusion at membrane interfaces (Schmid et al., 2016). These and other talks throughout the workshop highlighted the fact that, during complex tissue development, cells are integrating inductive as well as repulsive cues to generate the final morphology.

A majority of organs are planar polarized and discussions surrounding talks on planar cell polarity (PCP) not only questioned its role of during tissue development but also asked whether it has a role in maintaining tissue homeostasis. John Wallingford showed that PCP protein localization is highly dynamic in cells during morphogenesis but then stabilizes once the tissue has stabilized (Butler and Wallingford, 2017). However, epithelial wound healing recapitulates aspects of convergent extension, and Asako Shindo (Nagoya University, Japan) argued that PCP pathways are ostensibly redeployed after injury to promote repair. Furthermore, Carien Niessen (University of Cologne, Germany) provided mechanistic insights into epithelial polarity (Tellkamp et al., 2014) and showed that differential localization of epidermal growth factor (EGF) receptor directs junctional diversification in the epidermis. These talks and the related discussions throughout the workshop argue that we now have the tools and technologies to answer the fundamental question of how cells and tissues acquire essential shapes and functions during development.

The force is strong with this one – forces, form and function

An overarching focus of the meeting was on forces, form and function, and how these cooperate to regulate tissue dynamics. It is now generally accepted that the generation and interpretation of forces occurs during development and cancer, and that these can shape tissue morphogenesis. Despite this, much discussion was centred on how we define, measure and interpret forces in morphogenesis. This underscores that the field still has some fundamental, unanswered questions to address. For instance, what do people in the field actually mean when they describe processes as being mechano- and tension-sensitive? At what scale is this operating? And what is the consequence of a force in a tissue, if only changing the shape of something? How do different pools of cells within a tissue differentially sense force and undergo distinct morphogenetic processes within the same tissue? A number of discussion sessions and talks addressed these points.

Two important points were stressed by the audience with regard to defining how forces shape tissues. The first was made by Valerie Weaver (University of California, San Francisco, CA) who emphasised that molecules are in equilibrium. State changes are induced by the application of energy that, in turn, changes the system. This can be through force – i.e. mechanotransduction – or through chemical modification, such as phosphorylation. These processes are often considered distinct but, perhaps, should be reconsidered as different means to a common end. To this end, Alpha Yap (University of Queensland, Brisbane, Australia) described dynamic patterns of Src family kinase activation near areas of apoptotic cell extrusion from epithelial monolayers, which allow junctional relaxation and cell extrusion. Valerie Weaver provided evidence that force itself can act as a differentiation factor by modifying the commitment of pluripotent cells into different cell lineages (Przybyla et al., 2016). Indeed, culture of embryonic stem cells on matrices with differing stiffness induces the formation of primitive streak-like structures *in vitro*.

The second point was emphasized by Bénédicte Sanson (University of Cambridge, UK), who probed what we mean when we use terms such as cell adhesion or junctional tension *in vivo* – as we don't yet understand the precise mechanisms behind these processes and how these affect modes of movement in tissues. Alex Nestor-Bergmann (University of Manchester, UK) spoke about the development of mathematical methods in order to better understand the rules of how forces and tissue mutually shape each other. Notably, Alex highlighted that, by changing the shape-to-stress ratio of each cell, some unexpected emergent properties are observed, such as seemingly stress-bearing cell chains or veins in a tissue. These conclusions were reminiscent of those of Benedicte Sanson (University of Cambridge, UK), who described large multicellular actomyosin cables that help order polarized cell intercalations during body axis extension in *Drosophila* (Tetley et al., 2016). All of these discussions culminated in a simple question – perhaps the key question in developmental biology: how do cells sense which of them should do what?

The known unknowns – looking to the future

A number of speakers illustrated how improvements in technology, particularly in building imaging hardware and developing computational tools, have allowed a higher-resolution understanding of morphogenesis. But what type of imaging tool might allow us to address development and the heterogeneity of multicellularity? David Bryant (University of Glasgow and CRUK Beatson Institute, UK) described his approaches to develop high-throughput and machine-learning-assisted phenotype classification

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of 3D collective cell invasion from spheroids. Kees Weijer (University of Dundee, UK) spoke about their recent developments of light sheet microscopy-based approaches to image gastrulation (Rozbicki et al., 2015). Using 3D rendering, his group can now trace cell movements back from primitive streak-generating cells; this allows mapping of force vectors to unravel how spatiotemporal tissue forces give rise to tissue morphogenesis.

Peter Friedl described how intravital imaging has changed much of our previously held views of how cell invasion occurs, by presenting that *in vivo* cancer cells appear to invade predominantly as collective chains, mechanically expanding extracellular tunnels as the cellular chain moves through it. Paul Timpson (Garvan Institute of Medical Research, Sydney, Australia) presented the analysis of signalling pathways *in vivo* by using intravital imaging of Förster resonance energy transfer (FRET)-based sensors for Rho GTPase signalling pathways, and discussed how the *in vivo* dynamics of these pathways are dramatically different to the *in vitro* situation. Moreover, short-term stromal targeting of Rho signalling pathways can prime cancer cells for enhanced chemotherapy (Vennin et al., 2017). Finally, Scott Fraser (University of Southern California, Los Angeles, CA) described new approaches in his lab to allow multiplexed, high-resolution imaging of cellular, tissue, and organ dynamics and *in situ* hybridization (Cutrale et al., 2017). As we develop these tools with increased sophistication, an important notion as discussed by the participants is how we can connect all the various 'omic' approaches to bridge cell and developmental biology. It was agreed that this should be a major consideration for the future.

Another important issue not addressed above is the repertoire of molecules studied in the field. In many different studies of morphogenetic processes, a small number of core molecules, such as the classic Rho GTPases (Rac1, Cdc42 and RhoA) or myosin-II, make an appearance. Are these really the core factors, or does a 'repeat offender' merely represent sampling bias in what we study? To address this, John Wallingford has begun investigating the so-called 'ignore'-ome, a series of – as yet – poorly understood genes. What this approach will uncover is anyone's guess but, as agreed by all, one that is necessary and noble.

Conclusions

The success of a meeting can be measured by participants leaving with the feeling they have gained more than they contributed. We cannot speak for all participants but believe that this has, indeed, been the outcome of a meeting designed to foster openness and participation at all levels. It seems that some gentle force can cause a beneficial intellectual morphogenesis, too.

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