

Getting the measure of things: the physical biology of stem cells

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Summary

In July 2013, the diverse fields of biology, physics and mathematics converged to discuss 'The Physical Biology of Stem Cells', the subject of the third annual symposium of the Cambridge Stem Cell Institute, UK. Two clear themes resonated throughout the meeting: the new insights gained from advances in the acquisition and interpretation of quantitative data; and the importance of 'thinking outside the nucleus' to consider physical influences on cell fate.

Key words: Physical Biology, Quantitative biology, Stem cells

Introduction

How do cells make decisions, and how do they coordinate these decisions with their neighbours to build tissues? We have always known that development is a highly dynamic process, but there has been a tendency to take a rather static view of the way that extrinsic signals trigger differentiation decisions. In recent years, fluorescent reporters and real-time imaging technology have revealed to us that signalling and transcription are more dynamic than previously thought. The challenge now is to understand how stable patterning of embryonic and adult tissues emerges from these dynamic processes. The best way to tackle this question is through quantitative description of the molecular interactions that drive cell fate decisions. Here we face a problem: biology seems a messy and complicated business, so how can we measure the key parameters with sufficient accuracy and resolution? This was the one of the central themes of 'The Physical Biology of Stem Cells' meeting, organised by Austin Smith (Wellcome Trust Centre for Stem Cell Research, Cambridge, UK), Alfonso Martinez Arias (University of Cambridge, UK) and Ben Simons (University of Cambridge, UK). It was exciting to hear at this meeting how these problems are being overcome through the development of methods to quantify transcription and protein expression. This, combined with high-resolution imaging and modelling tools for interpreting this quantitative data, bring new insights into the ways that cells make decisions.

Another theme of the meeting was a shift away from thinking of stem cells as formless blobs passively converting extrinsic signals into transcriptional outputs. Cells experience various types of mechanical forces that could modulate the way that genes are expressed in response to differentiation cues, or even influence cell fate decisions independently of transcription. We heard of advances in techniques to directly measure and manipulate these forces. Other themes included the use of *in toto* quantitative imaging and multifactorial engineering to monitor and manipulate cell fate decisions *in vivo* and in cultured stem cells.

Measuring the dynamics of transcription and signalling

How can stable pattern be established in the highly dynamic context of a developing embryo? Several speakers described the use of fluorescent reporters for collecting quantitative data on transcription and signalling pathway activity at single cell resolution. Alexander Aulehla (EMBL, Heidelberg, Germany) looked at developmental patterning from the perspective of time rather than space, a concept that emerged from several talks in the meeting. Aulehla developed a novel *in vitro* assay using sensitive fluorescent reporters to directly measure oscillation dynamics in Notch activity during the process of mouse mesoderm segmentation. He showed evidence that the position and size of each segment depends on a slight shift in the phase of these oscillations from one cell to the next (Lauschke et al., 2013). Aulehla also discussed unpublished current work that addresses how the phase gradient is set up in the first place and how it is interpreted. Time was also a central theme for James Briscoe (NIMR, London, UK). He used a combination of fluorescent reporter measurement and computational modelling to describe the dynamic relationship between signal concentration and transcriptional response output in the patterning of the vertebrate neural tube: these quantitative data feed into a simple model that has impressive explanatory power. Briscoe explained how the dynamic relationship between signal and output could emerge through the influence of a simple transcriptional network that allows the output to adapt to changes in the signal input over time (Balaskas et al., 2012). Furthermore, as long as the network remains intact, then correct patterning can occur, even after genetic disruption of Shh signalling. This is an experimental observation that was previously difficult to explain but emerges clearly from the model. Further advances in signalling dynamics came from Holger Gerhardt (CRUK, London, UK). Gerhardt used advanced imaging techniques to make detailed measurement of the dynamic movements of endothelial cells during vascular development in mouse and zebrafish. Through an impressive combination of modelling and experimentation, Gerhardt showed how the signalling dynamics within this system can change dramatically in response to shifts in particular parameters, for example VEGF concentration, and how this can help to explain the disrupted vasculature seen in some tumours.

Feedbacks in transcription or signalling are not the only sources of dynamic behaviour. Michael Elowitz (Caltech, Pasadena, CA, USA) presented an intriguing new type of feedback mechanism in the haematopoietic system. With his collaborators Ellen Rothenberg (Caltech), Hao Yuan Kueh (Caltech), Stephen Nutt (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) and Ameya Champhekar (Caltech), he used a fluorescent reporter to measure the dynamic expression of the transcription factor PU.1 in a blood progenitor cells that become either macrophages (favoured by increased PU.1 expression) or B cells (favoured by a decrease in PU.1 expression). They found that the

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high levels of PU.1 in macrophages are due to an accumulation of this stable protein over the longer cell cycle of macrophages, rather than a change in transcriptional activity. Because PU.1 also regulates the cell cycle, this creates a 'cell-cycle coupled' feedback loop that seems able to confer greater stability on cell fate decisions than can be achieved through conventional transcriptional feedbacks alone (Kueh et al., 2013). Elowitz raised the intriguing speculation that similar mechanisms may operate in neural stem cells, where there is already evidence that cell cycle length can directly affect cell fate (Salomoni and Calegari, 2010).

Finally, it is important to remember that transcriptional dynamics may not reflect the dynamics of the resulting protein expression. Fabian Theis (Helmholtz Zentrum München, Neuherberg, Germany) reported on work, carried out in collaboration with Timm Schroeder (ETH, Zurich, Switzerland), using Nanog-GFP fusion reporters to directly quantify the dynamic behaviour of Nanog protein in embryonic stem cells (ESCs). By monitoring these dynamics in a very careful way, correcting for cell volume and cell cycle phase, Theis was able to extract information about when and how cells transition between different states. Interestingly, the model that emerged differs from the types of models evoked by others to explain the behaviour of reporters of Nanog transcription, highlighting differences between protein and mRNA dynamics that will be interesting to explore further.

Fluorescent reporters are powerful tools, but they do have their limitations when it comes to making accurate measurements of transcriptional events. Alexander Van Oudenaarden (MIT, Cambridge, MA, USA; Hubrecht Institute, Utrecht, The Netherlands) developed an RNA-FISH based method that allows precise measurement of transcription by counting mRNA molecules in individual cells (Itzkovitz and van Oudenaarden, 2011). He used this technique to tackle the issue of how the activator and repressor modes of *Gli2/3*, a mediator of Shh signalling, are balanced in order to achieve appropriate patterning. Accurate measurements of the outputs of Shh signalling feed into a mathematical description of these different modes of activity. This allows one to indirectly 'see' patterns in these activities, despite the fact that these activities cannot be directly visualised by conventional means because they reside in the same molecule, *Gli3*. Single-molecule RNA-FISH technology is emerging as a powerful tool that will transform the power of mathematical modelling in providing direct information about developmental processes.

How many events can we measure in a single cell?

Another important advance in recent years comes from our ability to simultaneously measure expression of a large number of genes over key developmental transitions at high temporal and cellular resolution. Julia Tischler (Gurdon Institute, Cambridge, UK) presented her work using quantitative single-cell PCR to investigate how individual stem cells acquire competence for the germ cell fate and to track the dynamic transcriptional changes of cell fate transitions *in vitro*. Jenny Nichols (CSCI, Cambridge, UK) performed the impressive technical feat of profiling the entire transcriptome from groups of fewer than a dozen cells taken from the individual inner cell mass of pre-implantation embryos. By homing in on the particular developmental window during which cells acquire naive pluripotency, she was able to identify candidate factors that may regulate this poorly understood event.

We are now getting used to the idea that the entire transcriptome can be profiled in an individual cell. By stark contrast, it seems impossible to measure more than a handful of proteins at single cell resolution because of the inherent limitations of conventional

fluorescence-based detection methods. This pessimistic vision has been overturned by Gary Nolan (Stanford University, CA, USA) who gave us a stunning glimpse of the future with his new method to measure at least 50, and potentially over 100, proteins in individual cells. The technique is analogous to flow cytometry, but uses transition metals and their isotopes, rather than fluorophores, to label antibodies, and mass spectrometry, rather than lasers, to detect and quantify these labels (Bodenmiller et al., 2012). The challenge, then, comes from how to interpret this huge quantity of data: in particular, how to extract dynamic information about cell fate decisions. Nolan presented modelling tools that reconstruct the trajectories followed by cells over time, setting up a framework against which it becomes possible to monitor changes in the rate or direction of travel along these trajectories in response to particular experimental conditions.

Another challenge in the field is how to gather reliable quantitative data on cell lineage *in vivo* without disrupting the dynamics of the system. For example, how can one track stem cells and their progeny in a tissue such as the gut that undergoes rapid turnover, without using lineage-labelling techniques that may inadvertently disrupt normal stem cell dynamics? Edward Morrissey (CRUK, Cambridge, UK) described an ingenious method, developed by collaborator Doug Winton (CRUK, Cambridge, UK), to label cells through use of a genetic reporter that is activated by a rare chance mutation event, thus avoiding the use of exogenous chemicals to trigger the labelling event. The frequency of labelling is low enough to allow the dynamic behaviour of individual stem cell-derived clones to be followed over time, in healthy gut or in intestinal adenomas. Through mathematical modelling of this continuous labelling data, it was possible to quantify the number of functional stem cells in crypts and adenomas. Contrary to previous reports, they found that significantly lower numbers of 'working' stem cells are present in the intestinal epithelium (five to seven stem cells per crypt) and in adenomas (nine stem cells per gland), and that those stem cells are also replaced at a significantly lower rate than previously thought.

An alternative way to monitor the dynamics of cell fate decisions is by directly observing and interrogating cells as they differentiate, but we are too often hampered by a lack of specific markers of the key transition states. Sally Lowell (MRC CRM, University of Edinburgh, UK) reported that the transcription factor *Tcf15* can be used to monitor the first steps that pluripotent cells take towards somatic lineage choice (Davies et al., 2013). She described a multifactorial quantification approach developed in her lab by Guillaume Blin to measure the orientation of individual cells with respect to their neighbours while at the same time monitoring differentiation using transcriptional reporters. This type of approach will help to address the issue of how topological organisation of cells might cooperate with transcriptional events to coordinate early cell fate decisions (Fig. 1).

Physical forces in stem cells and development

Robust development depends not only on transcriptional and signalling networks but also on higher-order feedbacks propagated through mechanical events such as strain and differential adhesion. This point was emphasised by Sean Megason (Harvard University, Cambridge, MA, USA). He presented a number of different examples to support this idea, making use of impressive *in toto* imaging techniques (Mosaliganti et al., 2012; Xiong et al., 2013) to quantify dynamic changes in cell shape and position in zebrafish embryos. In a similar vein, Yoshiki Sasai (CDB Riken, Kobe, Japan) presented his work on optic cup formation from both

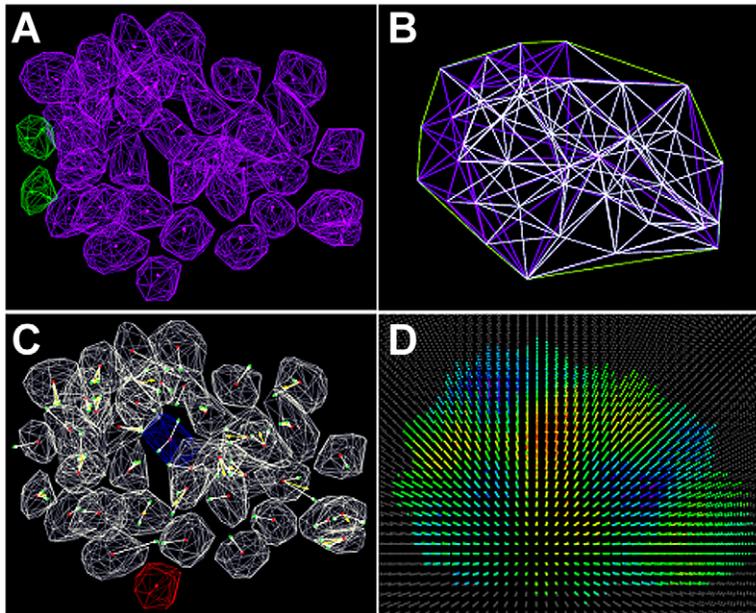


Fig. 1. Multifactorial quantification of transcriptional and topological properties of pluripotent cells using tools developed by Guillaume Blin (Lowell lab, MRC CRM, Edinburgh, UK). (A) Segmentation of nuclei and detection of cells expressing a Sox1GFP neural reporter (green). (B) Three-dimensional triangulation analysis to identify cells located at the rim (green), the hull (purple) or the inside (white) of the group. (C) Spatial orientation analysis, describing the intrinsic asymmetry of each cell. (D) Heat map showing degree of convergence of the cells based on the orientation analysis shown in C.

mouse and human ESCs. He used a combination of *in toto* imaging, atomic force microscopy and computational modelling to uncover the sequence of events that drive the remarkable ‘self-driven morphogenesis’ of the optic cup as it autonomously self-organises from ESCs (Eiraku et al., 2011; Nakano et al., 2012). He described interesting species differences in this process. In human, unlike in mouse, the convex shape of the optic cup does not depend on the constraint imposed by the stiffer outer ‘shell’ of the retinal pigmented epithelia, but is instead driven by the preferential localisation of nuclei to the apical side of the retinal cells. This imposes a wedge shape on each cell and a curvature on the tissue.

We heard from a number of physicists who are bringing new tools and new insights to developmental biology. Kevin Chalut (Cavendish Laboratory and CSCI, Cambridge, UK) presented data on some unusual mechanical properties that specifically characterise pre-committed cells, but not naive pluripotent cells or differentiated cells. By manipulating their physical environment in a microfluidic chamber, Chalut compared the physical properties of these cells with those of their descendants and antecedents in order to identify putative physical differences. The idea that cells dynamically alter their physical properties as well as their transcriptional state as they take the first steps towards lineage commitment helps broaden our thinking about the types of intrinsic changes that help to prepare cells for differentiation. Kristian Franze (Cambridge University, UK) highlighted the particular importance of physical forces in shaping the developing nervous system. Furthermore, he discussed a direct role for physical forces in visual message relay in ommatidia: the compound eye of arthropods. When exposed to light, ommatidia experience a physical contraction that is directly responsible for transducing information from the incoming photon to the responding ion channel (Hardie and Franze, 2012). It is evident from these studies that physical forces can influence cell behaviour, and that physicists are having an increasingly important influence on the field of stem cell biology.

How do cells sense and interpret these physical forces? Adam Engler (UCSD, San Diego, CA, USA) reported that mesenchymal stem cells use a ‘molecular strain gauge’ to control their

differentiation into muscle progenitors. Contractile force induces the deformation of vinculin (del Rio et al., 2009), changing its conformation to expose a cryptic binding site that binds and activates MAPK and consequently expression of the pro-differentiation transcription factor MyoD (Holle et al., 2013). This is a fascinating example of a mechanism that ‘tunes’ the activity of a signalling pathway in response to a particular physical environment. Ana Teixeira (Karolinska Institute, Sweden) described a ‘braille-like’ mechanism for sensing extrinsic information that depends on the ability of a cell to sense and interpret the spatial organisation of ligand/receptor complexes. She showed us an impressive new method for precise control over receptor spacing, using ‘nano-callipers’ to position Eph receptors at pre-defined positions. DNA origami technology (Han et al., 2011) is used to generate ‘pinboards’ to which Eph receptors can be attached at defined locations. This technology raises exciting possibilities for understanding in greater depth how stem cells interpret extrinsic cues during differentiation: it is possible that physical constraints may influence stem cell behaviour through their effects on the spatial distribution of ligand-bound receptors on the cell surface, and these new tools make it possible to perform manipulations at sub-cellular scales that will make it possible to test these ideas.

Manipulating the stem cell micro-environment

Stem cells usually reside in niches that provide chemical and physical cues to support appropriate self-renewal or differentiation of their resident cells. How can we capture the multifactorial nature of stem cell-niche interactions in order to better control stem cell behaviour in culture? Matthias Lutolf (EPFL, Lausanne, Switzerland) discussed his approach to this challenge, which combines biomaterials science with robotics and microfabrication to create a large range of different micro-environments based on synthetic hydrogels. He is able to vary combinatorially the mechanical properties and degradability of the substrate, as well as the array of matrix and signalling molecules presented within these gels (Gobaa et al., 2011). He described new work using Sox1GFP neural reporter ESCs to screen for an ‘artificial niche’ that supports optimal growth and neural differentiation of pluripotent cells in 3D.

One interesting conclusion from this work was that the physical constraint provided by the gel emerged as one of the most important influences on neural differentiation, being at least as important as soluble factors. This work highlights the importance of understanding the influence of physical forces experienced by cells as they differentiate, and applying this knowledge to help us to improve differentiation protocols. This idea was further explored by Shulamit Levenberg (Israel Institute of Technology, Haifa). By combining multiple cell types within specially engineered three-dimensional scaffolds, she can create a functional vasculature within pancreatic islets that helps these islets to survive, integrate and function after transplantation into mice.

Concluding remarks

Over 100 years have passed since Thomas Hunt Morgan, a founding father of developmental biology, predicted, 'we will never understand the phenomena of development and regeneration' (Berrill, 1983). We have come a long way since then, thanks in large part to the power of developmental genetics, but there is still much that puzzles and intrigues us. The field is entering an exciting new phase that will allow us to measure multiple events at impressively high resolution in time and in space. At the same time, rigorous analytical tools are being developed that allow us to extract meaningful information from these quantitative data. This will surely push us closer to a more complete understanding of the web of interactions, both genetic and physical, that shape the beautiful complexity of multicellular organisms.

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Competing interests statement

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References

- Balaskas, N., Ribeiro, A., Panovska, J., Dessaud, E., Sasai, N., Page, K. M., Briscoe, J. and Ribes, V. (2012). Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell* **148**, 273-284.
- Berrill, N. J. (1983). The pleasure and practice of biology. *Can. J. Zool.* **61**, 947-951.
- Bodenmiller, B., Zunder, E. R., Finck, R., Chen, T. J., Savig, E. S., Bruggner, R. V., Simonds, E. F., Bendall, S. C., Sachs, K., Krutzik, P. O. et al. (2012). Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. *Nat. Biotechnol.* **30**, 858-867.
- Davies, O. R., Lin, C.-Y., Radzsheuskaya, A., Zhou, X., Taube, J., Blin, G., Waterhouse, A., Smith, A. J. H. and Lowell, S. (2013). Tcf15 primes pluripotent cells for differentiation. *Cell Rep.* **3**, 472-484.
- del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J. M. and Sheetz, M. P. (2009). Stretching single talin rod molecules activates vinculin binding. *Science* **323**, 638-641.
- Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T. and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472**, 51-56.
- Gobaa, S., Hoehnel, S., Roccio, M., Negro, A., Kobel, S. and Lutolf, M. P. (2011). Artificial niche microarrays for probing single stem cell fate in high throughput. *Nat. Methods* **8**, 949-955.
- Han, D., Pal, S., Nangreave, J., Deng, Z., Liu, Y. and Yan, H. (2011). DNA origami with complex curvatures in three-dimensional space. *Science* **332**, 342-346.
- Hardie, R. C. and Franze, K. (2012). Photomechanical responses in *Drosophila* photoreceptors. *Science* **338**, 260-263.
- Holle, A. W., Tang, X., Vijayraghavan, D., Vincent, L. G., Fuhrmann, A., Choi, Y. S., Del Álamo, J. C. and Engler, A. J. (2013). In situ mechanotransduction via vinculin regulates stem cell differentiation. *Stem Cells*. doi: 10.1002/stem.1490
- Itzkovitz, S. and van Oudenaarden, A. (2011). Validating transcripts with probes and imaging technology. *Nat. Methods* **8** Suppl., S12-S19.
- Kueh, H. Y., Champhekar, A., Nutt, S. L., Elowitz, M. B. and Rothenberg, E. V. (2013). Positive feedback between PU.1 and the cell cycle controls myeloid differentiation. *Science* **941**, 670-673.
- Lauschke, V. M., Tsiairis, C. D., François, P. and Aulehla, A. (2013). Scaling of embryonic patterning based on phase-gradient encoding. *Nature* **493**, 101-105.
- Mosaliganti, K. R., Noche, R. R., Xiong, F., Swinburne, I. A. and Megason, S. G. (2012). ACME: automated cell morphology extractor for comprehensive reconstruction of cell membranes. *PLoS Comput. Biol.* **8**, e1002780.
- Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M. and Sasai, Y. (2012). Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* **10**, 771-785.
- Salomoni, P. and Calegari, F. (2010). Cell cycle control of mammalian neural stem cells: putting a speed limit on G1. *Trends Cell Biol.* **20**, 233-243.
- Xiong, F., Tentner, A. R., Huang, P., Gelas, A., Mosaliganti, K. R., Souhait, L., Rannou, N., Swinburne, I. A., Obholzer, N. D., Cowgill, P. D. et al. (2013). Specified neural progenitors sort to form sharp domains after noisy Shh signaling. *Cell* **153**, 550-561.