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INTERVIEW

The people behind the papers – Nicole Repina and David Schaffer

During embryogenesis, cells differentiate and organise into spatially defined regions in response to varying patterns of signalling. A new paper in Development uses an optogenetic system (optoWnt) to investigate how cells organise into distinct domains in response to Wnt signalling in two-dimensional human embryonic stem cell cultures. To hear more about the story behind the paper, we caught up with the first author Nicole Repina and her PhD supervisor David Schaffer, Professor at University of California, Berkeley.

Nicole Repina (L) and David Schaffer (R)

David, can you give us your scientific biography and the questions your lab is trying to answer?

DS: I did my training at Stanford University (BS), MIT (PhD) and the Salk Institute (postdoctoral fellowship) at the interface of chemical engineering and molecular and cell biology, with a focus on neuroscience. The engineer in me is highly interested in developing new technologies that can enable quantitative investigation of how biological systems and behaviours are regulated. We apply these approaches to study the signalling mechanisms by which stem cells, from early development to the adult nervous system, make fate decisions and pattern tissues.

Nicole, how did you come to work in David's lab, and what drives your research today?

NR: During my bachelor studies I worked on studying tissue regeneration in different multicellular systems. I particularly enjoyed microscopy-based quantification, so for my PhD I wanted to deepen my knowledge of optical engineering. I was intrigued by optogenetics and the combined engineering and biological approach of David's lab, where I had the freedom to both develop optical tools for light patterning and apply them to interesting questions in stem cell biology. Today, I'm continuing to work on understanding the molecular mechanisms for cell self-organisation and tissue-scale patterning using microscopy techniques in 3D systems, during my postdoctoral work in the lab of Prisca Liberali in Basel, Switzerland.

Can you give a short introduction to the OptoWnt system used in this paper?

DS: Nearly 15 years ago, we developed a strong interest in developing technologies to perturb cellular signalling pathways with precise quantitative and temporal control. At the time, optogenetics based on light-responsive ion channels was already a powerful and established approach in neuroscience, but there was a comparative lack of technologies for controlling growth factor, morphogen or cytokine signalling pathways. A highly talented graduate student in our lab, Lukasz Bugaj (now faculty at the University of Pennsylvania), discovered that the *Arabidopsis* protein Cryptochrome 2 (Cry2), which was known to heterodimerise in response to light, underwent rapid and reversible self-oligomerisation upon blue light illumination in mammalian cells. There are many pathways in which self-oligomerisation of a signalling protein is necessary and sufficient for signal activation, so we began to fuse Cry2 to a variety of signalling proteins and were thereby able to place Wnt, RhoA, Cdc42 and receptor tyrosine kinase signalling under light control for the first time.

Can you give us the key findings of the paper in a paragraph?

NR: The first key finding is that we are able to activate canonical Wnt signalling optogenetically in human embryonic stem cells (hESCs). This means that when we illuminate these optoWnt-expressing hESCs with blue light, we activate Wnt and induce cell differentiation to mesendoderm and an epithelial-to-mesenchymal transition. Second, when we grow the optoWnt light-responsive cells in co-culture with wild-type cells, where the two cell types are well mixed in epithelial colonies, we find that the optoWnt cells spatially segregate and form distinct domains from the wild-type cells. Interestingly, this population-level self-organisation emerges in response to optoWnt stimulation at the cellular level. We find that the self-organisation process is dependent on downstream activation of TGFβ signalling in optoWnt cells and on the cell state and motility changes associated with the epithelial-to-mesenchymal transition. Lastly, we find that the optoWnt/wild-type co-culture system is useful for studying signalling feedback between the epithelial and mesendodermal cell populations, which also models the spatial arrangement of cell populations within the gastrulating embryo.

Were you surprised to find that, in the optoWnt/wild-type co-culture system, self-organisation can occur without a localised source of Wnt signalling?

DS: We initiated the co-culture work to investigate how differentiation of one subpopulation of cells (through activation of Wnt) could alter the fate of the other subpopulation, but we did not anticipate that the cells would spatially self-organise. This finding motivated a strong interest in using 2D and, in the future, 3D models to investigate how symmetry breaking and self-organisation can occur even in the absence of a spatially asymmetric cue. We greatly

NR: Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland.
DS: Department of Bioengineering, Department of Chemical and Biomolecular Engineering, Helen Wills Neuroscience Institute and Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA.
E-mail: schaffer@berkeley.edu; nicole.repina@fmi.ch

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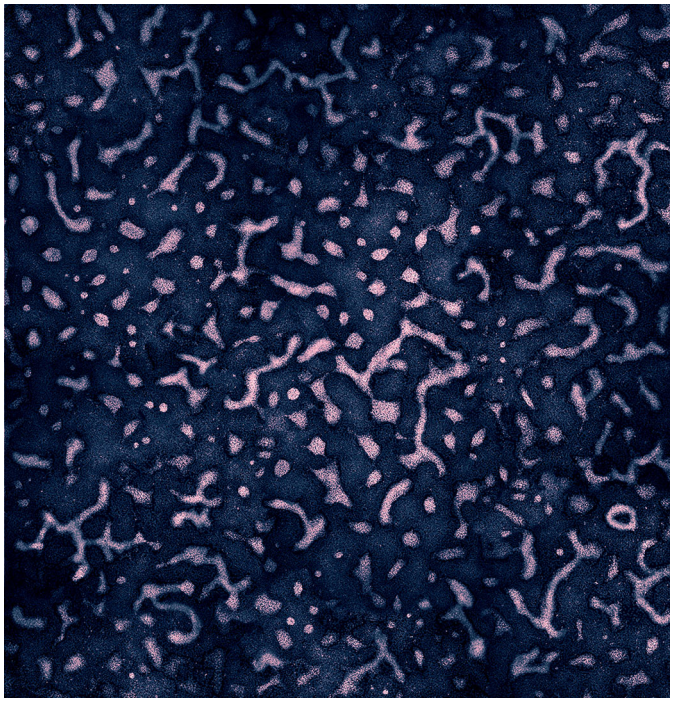

Human embryonic stem cells self-organising into epithelial (dark blue) and mesenchymal (pink) domains upon optogenetic Wnt stimulation. Montage of confocal microscopy images of cell nuclei stained with DAPI.

look forward to continuing to explore this concept of organismal development with the aid of new technologies.

What implications will your study have on understanding cell self-organisation in early development?

DS: The process by which a single human cell develops into a collection of >30 trillion intricately ordered cells involves a complex series of symmetry breaking events. It is well known that a spatially ordered external cue can organise a tissue, and that cell sorting can also contribute to order formation, though new technologies can enable a deeper investigation of both mechanisms. Our experimental system indicates that cell-to-cell variation in responsiveness to a spatially uniform Wnt cue, followed by cell migration and sorting, leads to symmetry breaking. Furthermore, transcriptomic analysis of cellular subpopulations during this process enables elucidation of the intracellular and intercellular signalling mechanisms underlying this behaviour. We hope that this model system can be further developed to study symmetry breaking at various stages of early human development.

Nicole, was there any particular result or eureka moment that stuck with you when doing the research for this paper?

NR: I remember when I first saw the segregation of optoWnt and wild-type co-cultures under the microscope. The separation of the domains was so striking, with the optoWnt 'islands' forming long, snaking patterns meandering through the well. It reminded me of aerial photos of river deltas cutting through some lush tropical landscape. I was in awe and just taking images of different fields-of-view and magnifications to understand what I was seeing. To me, these unexpected moments are the gems that make research beautiful.

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How about the flipside, any moments of frustration or despair?

NR: Our first hypothesis was that segregation was occurring due to differential adhesion of the optoWnt and WT populations. I spent almost a year generating dozens of knockdown lines of the various adhesion proteins expressed in our system, yet none of them yielded a strong phenotype or decreased the cell segregation. We think this is because there is redundancy between the different adhesion molecules, so likely combinatorial knockdowns are necessary.

David, where will this story take your lab next?

DS: Nicole originally observed this cell sorting behaviour in 2D. This is motivating us to investigate how spatially homogeneous signals can lead to self-organisation in 3D, as a closer model for organismal development. This work includes human systems that are emerging as early models of gastrulation, with the emergence of all three germ layers, as well as early neural tube formation.

Finally, let's move outside the lab, what do you like to do in your spare time?

DS: I love travel, wine and great food with friends and family, and we're having fun making up for time lost during the pandemic. I also greatly enjoy staying in shape by rock climbing, which is essentially Newton's laws of motion on a wall.

NR: Outside of the lab, I love spending time in nature in the company of family and friends, be it on an alpine summit, in the garden with my cat, or a picnic by the river. I enjoy cooking, reading and all sorts of mountain activities like climbing, mountaineering and ski touring.

Reference

Repina, N. A., Johnson, H. J., Bao, X., Zimmermann, J. A., Joy, D. A., Bi, S. Z., Kane, R. S. and Schaffer, D. V. (2023). Optogenetic control of Wnt signaling models cell-intrinsic embryogenic patterning using 2D human pluripotent stem cell culture. *Development* **150**, dev201386. doi:10.1242/dev.201386