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FIRST PERSON

First person – Andrew Porter

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Andrew Porter is first author on 'The interaction between CASK and the tumour suppressor Dlg1 regulates mitotic spindle orientation in mammalian epithelia', published in JCS. Andrew is a postdoc in the lab of Prof. Angeliki Malliri at the Cancer Research UK Manchester Institute, investigating how abnormal mitotic events – spindle misorientation, centriole defects and chromosomal instability – contribute to tumourigenesis.

How would you explain the main findings of your paper in lay terms?

Many tissues – including those in the human kidney and breast – are composed of a layer of epithelial cells surrounding a hollow lumen. When these cells divide, the newly-formed daughter cells usually sit side-by-side, maintaining a single layer of cells. In some cancers, however, these divisions are upside down, so that cells divide into the centre of the lumen and form disrupted structures. Control of the direction of these divisions depends on correctly positioning the cell division machinery within the cell. We found that Dlg1, a protein important for this process, needs to bind to its partner protein, CASK, to get to the right place in the cell. When we blocked this interaction, or made a version of Dlg1 that couldn't bind to CASK, the cell division machinery was misplaced, epithelial cells could no longer divide in the correct orientation, and formed 3D structures resembling early stages of some cancer types.

Were there any specific challenges associated with this project? If so, how did you overcome them?

We had seen misoriented divisions in fixed cells depleted for CASK or Dlg1, but as cell division is dynamic we wanted to observe this process in live cells in 3D cysts. We could do this in one or two cysts at a time on a conventional confocal microscope, but needed to observe many divisions for quantification. Working with Steve Bagley in our imaging facility, we developed a method to use the Opera Phenix High Content imaging platform to perform live imaging in a 96-well plate format, observing many cysts in multiple conditions in one experiment. Early attempts were unsuccessful as the cells were not dividing, probably due to phototoxic stress. Reducing the laser power and increasing the time interval helped, but the cysts were still not growing properly. We realised that the wells were slightly drying out over time. Eventually we found that something as simple as completely filling every spare well (and even the spaces between the wells!) with media finally prevented this, allowing us to image for up to 48 h and observe many misoriented divisions in knockdown cells.

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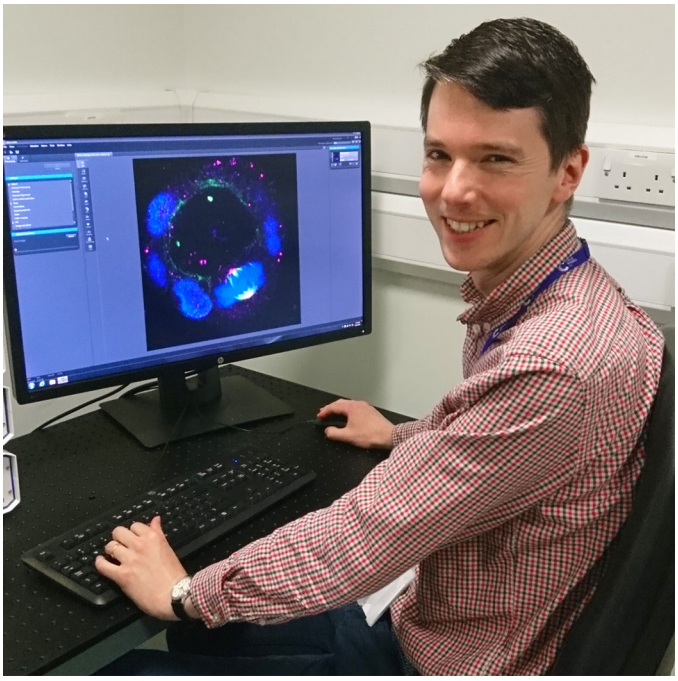

Andrew Porter

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

Early on in this project, we were thinking about disrupted 3D growth as a defect in cell polarity; cells with disrupted polarity might grow on top of or around each other, causing the multilumen phenotype we associated with CASK knockdown. However, when we looked at individual cells in multilumen cysts, they retained normal apical–basal localisation of marker proteins, so this option seemed unlikely. Similarly, there seemed no change in apoptosis (my next preferred hypothesis). I started reading papers about spindle orientation, and the effect this could have on 3D growth. Even so I was very sceptical that this was what was causing defective growth in CASK-depleted cysts, but did the experiments anyway to look at the angle of cell division. I was very surprised – and pleased! – when the results showed misoriented divisions, and this finding shaped the whole direction of the project.

Why did you choose Journal of Cell Science for your paper?

We chose Journal of Cell Science because of its strong reputation in the field of cell biology, and also because of its clear and positive policies of welcoming preprints. This paper is the first preprint from our lab, as we wanted to share our preliminary findings and get feedback from the scientific community, and it was very straightforward to submit from bioRxiv directly to Journal of Cell Science.

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

My undergraduate tutor, Alison Woollard, was a big factor in encouraging me to pursue a PhD, and her enthusiasm for research was infectious. My PhD supervisor, Stephen Nurrish, taught me

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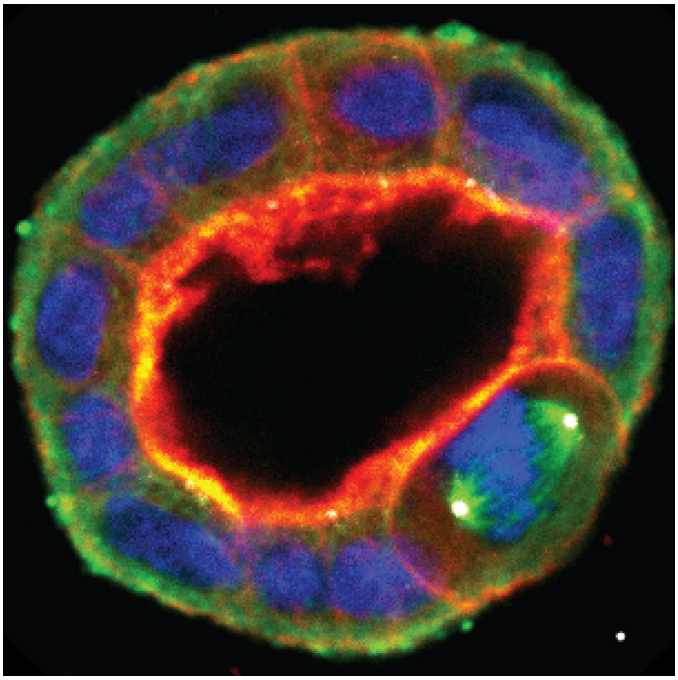

A mitotic cell (bottom right) dividing parallel to the lumen of an MDCKII cyst (marked with strong actin staining, orange), showing the mitotic spindle stained for tubulin (green), centrosomes (white) and aligned chromosomes (blue).

how to give clear presentations, and how to restructure ideas as new data becomes available. Having worked with *C. elegans* for my PhD I will always be indebted to the help of my co-author, Natalie Mack, who kindly took the time to get me up-to-speed with cell biology techniques, and introduced me to 3D culture methods. And from my current supervisor, Angeliki Malliri, I've learnt so much about writing papers and grants, and benefited from her advocacy in many ways over the years.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

I have a vivid memory of being shown an electron micrograph of an animal cell in an A-level biology class, full of structure and detail. This was in contrast to the 'white circle with a blue dot in the middle' picture which we'd been taught for GCSE! I was blown away by the thought that there was this whole world inside every cell, and I remember thinking at that point that I wanted to know how it worked.

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What's next for you?

I'm currently working on another cell division project looking at the role of the Rac1-specific GEF Tiam1 at centrosomes. This builds on our previous findings and I'm excited to be using super-resolution techniques to explore the centrosome structure in greater details, as well as writing funding applications with the aim of becoming an independent researcher.

Tell us something interesting about yourself that wouldn't be on your CV

I'm a big fan of puns, and I also play the guitar, so I like re-writing songs with a science-related theme, such as to celebrate a viva defence or papers being published from the lab. Two of my favourites so far are 'Take My STEF Away' and 'Yap Pathway' (to the tune of 'Yesterday').

Reference

Porter, A. P., White, G. R. M., Mack, N. A. and Malliri, A. (2019). The interaction between CASK and the tumour suppressor Dlg1 regulates mitotic spindle orientation in mammalian epithelia. *J. Cell Sci.* **132**, 230086. doi:10.1242/jcs.230086