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FIRST PERSON

First person – Nicola Stevenson and Ian White

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. Nicola Stevenson and Ian White are co-first authors on 'Clathrin-mediated post-fusion membrane retrieval influences the exocytic mode of endothelial Weibel–Palade bodies', published in this issue of Journal of Cell Science. Dr Stevenson is a Research Associate at the University of Bristol, UK, investigating mechanisms of collagen trafficking, and Dr White is an electron microscopist at the MRC Laboratory for Molecular Cell Biology at University College London, UK.

Nicola Stevenson

How would you explain the main findings of your paper to non-scientific family and friends?

NS: Weibel–Palade bodies (WPBs) are storage granules that package up proteins ready for delivery to the cell surface upon receipt of a signal. These granules are found inside the cells lining your blood vessels and contain proteins required for blood clotting and inflammation.

In response to injury, WPBs migrate to the cell surface where their encapsulating membrane fuses with the cell surface membrane. This releases the granule contents into the blood vessel to initiate blood clot formation, as desired, but concomitantly increases the cell surface area as the two membranes become continuous. To counteract this, the cell must then internalise an equivalent amount of membrane to maintain its size. In our paper, we show that this is achieved by pinching the entire fused WPB membrane off from the cell surface back into the cell, creating an empty bag which can then be degraded. While this is happening, smaller bits of membrane are also retrieved directly from the fused granule membrane, indicating that specific areas of the membrane are recycled more quickly than the whole. These rapid recycling events remove the protein machinery used for fusion; if it fails, other WPBs inside the cell start to fuse with the old WPB membrane instead of fusing with new sites on the cell surface. This causes the clotting proteins inside to become knotted and trapped, impairing their release from the cell and their ability to function.

IW: Your WPBs hang around in the cells that line your blood vessels like organelle first responders, just waiting for their chance to shine when damage occurs. They're packed with huge amounts of a protein called von Willebrand's factor (vWF), organised as a furled up 'string'. When the cell is damaged, the WPBs spring into action, joining their own outer membrane to that of the cell in a process called exocytosis and spewing out their long strings of vWF into the blood flow. These act in several ways, linking and meshing together with strings from other WPBs both from the same cells and other damaged cells, thereby slowing the blood flow. The vWF strings specifically grab platelets from the bloodstream to further aid the formation of clots and stop the blood from leaking out of your blood vessels, where it belongs, and either into the body cavity or onto the floor, where it doesn't.

The vWF isn't everything however; the membrane of the WPB also carries important content, and when it joins to the outer membrane of the cell it allows that content to go about its business too. However, some of the items being delivered to the cell membrane need retrieving, and some of the cell's other proteins that assist the joining of the WPB to the cell membrane also need retrieving. They've been crucial in making the delivery, but like a driver and their van, they have business elsewhere rather than remaining in your driveway. If those crucial parts of the cellular machinery aren't able to leave the membrane, then they are unable to help other processes occur, or other WPBs deliver their cargo. In these cases, the new deliveries are unable to join to the cell membrane, but end up joining onto the exiting WPB that is still attached to it, akin to a second delivery van delivering to the first one that is still stuck in your drive rather than delivering directly to your door. This is hugely inefficient, and trying to deliver through an existing WPB is problematic. Our work helps to elucidate how the cell goes about the retrieval of crucial elements from the cell membrane rapidly following WPB release, maintains cell surface area, membrane composition and the efficient rescue of elements required for continued response to the emergency.

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Ian White

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

NS: The challenges of this project were largely technical – finding a way to observe the fate of such a small, dynamic piece of membrane in a time-resolved manner was particularly difficult. We knew from previous publications that the granule membrane was likely to be retrieved whole but despite trying out several live-labelling techniques, this was difficult to prove until we turned to correlative light-electron microscopy with the help of Ian. In the end, this was fortunate as it allowed us to see the small recycling events occurring on the fused WPB membrane, which was a more exciting find, and allowed us to develop a model for the regulation of exocytic mode.

IW: There is a huge amount of electron microscopy in this paper, almost none of it routine. There is correlative light and electron microscopy at the organelle level, and immunogold labelling and consecutive serial sectioning through whole cells. Electron microscopy is famously not a high-throughput or rapid technique, and consequently there are a lot of lab hours in this paper simply in electron microscopy time from sample prep and technically demanding sectioning. Then there is hunting for events that are fairly small and relatively infrequent in terms of the whole cell volume, when you are effectively subdividing that cell into 50–70 slices of only 70 nm thick. Fortunately, we have a great deal of experience with these techniques and a well-integrated approach to light and electron microscopy, which allowed us to be so ambitious in our ultrastructural approach.

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

IW: Buzz Baum, one of the Professors here at the MRC LMCB, recently wrote about this in a blog where he said: "…like a rock-climber, you need to get satisfaction from finding a good new foothold. Not just when surveying the view from the top." In agreement with this, my eureka moments are commensurately small but no less satisfying for it. With so much electron microscopy, it's almost a slow reveal rather than a eureka anyway, but when you move from section to section finding and imaging the area of interest, gradually building up a picture of an exocytic site, and "Yes!" there's a clathrin-coated vesicle budding off it, or maybe what appears to be another WPB fused to it. When you see that you know it's another new foothold in scaling the heights. It's nice to survey the view from the summit of having our paper published in Journal of Cell Science, but each of those new handholds and footholds en route were also significant achievements in their own right.

> "(…) my eureka moments are commensurately small but no less satisfying for it"

What's the most important piece of advice you would give first-year PhD students?

NS: Make sure you don't burn out! A PhD is a marathon not a sprint and there will be a lot of challenging times when things aren't going your way. A good work–life balance will help you keep perspective, promote ideas and creativity and ensure that you continue to enjoy science.

IW: It's not all about working hard (whatever your supervisor says!), it's about working efficiently. Don't go crazy on the world's biggest experiment until you have concluded that it's the best use of energy and resources. It's very easy for early enthusiasm to fade into despair if you've put all your energy in, worked really hard and gained little for it. Better to wait until you understand your system well, and have preliminary data showing the right course before you try and push the killer experiment. The time to work all hours will come later in your PhD believe me, but you will be more equipped and experienced to handle it and you will achieve more in a couple of days at that stage than you may do in several weeks at the start of your project.

> "A PhD is a marathon not a sprint and there will be a lot of challenging times when things aren't going your way."

What changes do you think could improve the professional lives of early-career scientists?

NS: I think longer post-doc contracts of 4–5 years should become more mainstream to give young researchers time to really explore and pioneer new techniques and approaches, and provide the opportunity to take on more risky projects. Depending on the subject, projects are often only coming together at the end of three years, especially if they require the development of new tools, and the personal pressure to publish in this time means the research cannot reach its full potential. With more time to develop ideas, the impact of the work will improve, as will the profile of the researcher.

IW: Stability. Everyone wants and needs stability. Three-year contracts drive more people out of academic science than anything else, in my opinion.

What's next for you?

NS: I completed the research published in this paper during my PhD at the LMCB in London and am now working at the University of

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Bristol as a Research Associate investigating the mechanisms of collagen trafficking with Prof David Stephens.

IW: There's always more electron microscopy to be done!

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

NS: In addition to working as a Research Associate, I moonlight as a gymnastics coach twice a week.

IW: My teammate and I came third in the first National Aeroball Championships at the Garden Festival in Ebbw Vale, Wales in 1992.

Reference

Stevenson, N. L., White, I. J., McCormack, J. J., Robinson, C., Cutler, D. F. and Nightingale, T. D. (2017). Clathrin-mediated post-fusion membrane retrieval influences the exocytic mode of endothelial Weibel–Palade bodies. *J. Cell Sci.* **130**, 2591-2605.