Peter Cullen: Nexins have it sorted

Cullen studies protein sorting in the endosomal network.

The endosomal network receives vesicles from the Golgi apparatus and the cell surface and then sorts and repackages their contents for recycling or export to other cellular destinations. Proteins of the sorting nexin family are central to this process. Some sorting nexins shepherd cargoes, others give shape to the endosomal tubules and vesicles, and yet others have functions that are still unknown.

Binding of sorting nexins to the lipid phosphatidylinositol-3-monophosphate (PI3P) localizes them to the endosomal network. An affinity for phosphoinositides and the proteins that bind them is also what brought Peter Cullen to study the endolysosome system and sorting nexins. With his extensive expertise in inositide biology (1, 2), Cullen is working to map the endosomal network and the activities of sorting nexins (3–5), as we learned when we called him at his lab at the University of Bristol.

IN THE FIELD

Are you originally from England?

Yes. I grew up on a small farm, about 60 or 70 acres, in Norfolk. I grew up picking fruits and driving tractors, combine harvesters, and other agricultural machinery with my brother, who's four years older than me.

But when I was growing up small farms like ours were being swallowed up by bigger landowners. My dad recognized that farming the land just wasn't going to be a viable option in the future, so he told my brother and I that we could do whatever we wanted except become farmers. Both my brother and I did degrees in biochemistry at the University of East Anglia.

> "I came away realizing that I wanted to do a postdoc in inositide signaling."

Did you have time for other interests besides helping out on the farm?

I've always been interested in and active in sports. I used to play lots of cricket when I was in school. My summers used to be spent either working on the farm or playing cricket for the local team. Also, growing up on a farm, I was always interested in wildlife, so I did lots of bird watching. Particularly after I finished up at university, I spent a lot of time walking the marshes and traveled around the country looking for rare migrants. Indeed, I have retained a great interest for observing wildlife and in particular animal behavior.

Do you still play cricket?

I took a break for about 15 years when I was working to get my lab going and when my daughter arrived. Then my daughter started to play for the local girls' team where we live in Bristol, and word got out that I used to play university-grade cricket, so I've recently been brought back into it. You play cricket for five or six hours, and then both teams adjourn to the bar and discuss life over a few drinks. It's a good way to spend a Saturday afternoon. The rest of my weekends are usually taken up with acting as a taxi service for a teenage girl. [Laughs]

LOVE FOR LIPIDS

You did your PhD with Alan Dawson…

I remember being in a third-year undergraduate lecture when Alan came to speak about inositide signaling. This was when the field was just beginning to blossom, and Alan raised a number of interesting questions about the field. That block of lectures made me decide I wanted to do my PhD in some form of inositide signaling, and I decided to join Alan's lab in Norwich. The overall aim of my PhD project was to try to describe how IP_3 stimulated calcium release. But in another part of that project I was working in collaboration with Robin Irvine on how another inositol phosphate called IP_4 regulates cellular calcium homeostasis.

PHOTO COURTESY OF ANISHA CULLEN

Peter Cullen

Near the end of my PhD I went to a Harden Conference on inositide lipids, and I came away realizing that I wanted to do a postdoc in inositide signaling in Robin's lab at Cambridge. Robin said he'd be glad to take me but funds were tight, so if I wanted to go I would have to get my own money. So I applied for a fellowship that fortunately got funded, and I was able to join his lab.

I had proposed to purify the IP_4 receptor using a radiolabel assay, but it took me about two years to establish a protocol for the isolation of enough radiolabeled IP_4 to do the assay. My breakthrough came after Robin met Kalwant Authi at a meeting. Kalwant worked on platelet homeostasis and had established a method for the separation of plasma membrane from other cellular membranes. In analyzing these samples it became obvious that platelets were chock-full of IP_4 binding activity. That was the critical step, because then I could go to the pig abattoir up the road and get hold of 100 liters of blood, purify out the platelet fraction, put it down various columns, and ultimately purify out the IP_4 receptor. That became amusing because Len Stephens and Phill Hawkins were also in Robin's lab at the time and were trying to purify PI 3-kinases from pig's blood. They started using around 700 liters for their work, so there was blood everywhere. It was quite grim at times [Laughs].

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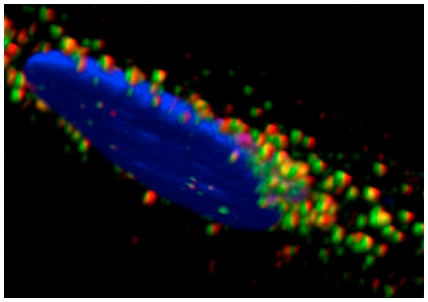

IMAGE COURTESY OF DR. MIRIAM BUJNY

A 3D-rendered, tilted view of endosomes stained for endogenous sorting nexin-2 (green) and the retromer component VPS26 (red). The nucleus is blue.

Nonetheless it was an amazing period in Robin's lab, with Len and Phill and so many other interesting people there.

You did eventually succeed in purifying the IP_4 receptor…

When we got the sequencing back from the purified protein, it turned out that it was a pleckstrin homology domain-containing protein that also had Ras GAP (GTPase activating protein) activity. We gave it a horrible name, $GAP1^{IP4BP}$—which doesn't roll off the tongue particularly well—and showed that its Ras GAP activity was regulated by binding IP_4. We published that work in *Nature*, and when I left to set up my own lab here at Bristol Robin let me take the entire project with me.

At first I just wanted to know what other proteins were regulated by IP_4. We identified three other proteins that were all members of the GAP1 family, and we studied their function and localization in cells. One really beautiful experiment we did was to show that two proteins—one called CAPRI and another called RASAL—could only bind to phospholipids in the presence of calcium. We further found that RASAL sensed the frequency of calcium oscillations that occur upon agonist stimulation, whereas CAPRI senses the amplitude of the oscillation.

NEXT, NEXINS

How did you first start working on sorting nexins?

In the early summer of 2001 a series of papers came out about a new lipid-binding protein domain called the PX domain, or phox homology domain. It's a binding module for various phosphoinositides including PI3P, a phospholipid that is enriched on early elements of the endocytic network. We were interested in the idea that this PX domain could function to target proteins to endocytic membranes and hence that characterizing these proteins may provide a route towards a molecular understanding of endosomal sorting.

I was fortunate to have an exceptional PhD student by the name of Jez Carlton whose project was to clone out the various PX domain-containing proteins in the human genome, including the sorting nexin family, and then tag them with GFP and look to see where they localized and how they behaved in a cellular context. One of the things that Jez noticed early on was that most of the nexins that we looked at were present on early endosomes but that a certain set of them, the SNX-BARs, appeared to tubulate the membrane under live-cell conditions. That was interesting because the endocytic network generates a tubular extension called the tubular endosomal network, or TEN, but it wasn't known how this happened.

> "We'd like to look at [this] in a global, unbiased way."

What features of SNX-BARs are needed to induce tubulation?

We very much feel that the SNX-BARs are able to sense membrane curvature, but in a concentration-dependent fashion they can also drive membrane tubulation. The SNX-BARs have a banana-shaped BAR domain and an amphipathic helix, both of which are required to induce membrane curvature, but they also form a higher-order lattice that lets them coat the tubule and help the tubule to grow. We don't understand how the SNX-BAR helical assembly actually forms, and that's one of the challenges that we want to try to address.

Not all nexins have BAR domains…

That's right. All nexins have PX domains, but it does not follow that every protein that's called a sorting nexin has to be involved in endosomal sorting. In fact, there is evidence that some of these proteins are also involved in endosomal signaling. There is data coming out, for example, that sorting nexin-27 is a member of the SNX-PX-FERM–like family of sorting nexins. Those proteins can also bind active Ras, and the argument is that they sense Ras in a GTP-bound state but only when it's on the endocytic system and that this may help regulate their activity in cargo sorting. Obviously, given our historical interest in Ras, this is something we're interested in looking into.

SNX27 also contains a PDZ domain that interacts with a specific set of PDZ binding motifs present on the cytosolic face of a number of cargoes. We think this allows SNX27 to capture these cargoes and stop them going down the lysosome or degradative pathway. We'd like to look at endosomal sorting in a global, unbiased way and follow all of the cargoes we can, and we're developing quantitative proteomic methods to do that. We've also become interested in next generation sequencing because we'd like to identify which sorting nexins, and which of their associated proteins, are involved in specific human diseases.

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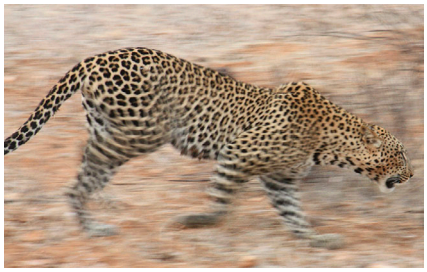

IMAGE COURTESY OF PETER CULLEN

Cullen snapped this image of a hunting leopard in Kenya's Samburu National Reserve.