People & Ideas

Sean Munro: Revealing the Golgi's true identity

Munro studies how proteins localize within the secretory pathway.

Although cargo proteins move through the different organelles of the secretory pathway, other proteins remain in place to give each compartment its own unique identity and function. Sean Munro, from the MRC Laboratory of Molecular Biology in Cambridge, England, has been interested in how proteins find their place within the secretory pathway since his PhD studies with Hugh Pelham, when he discovered a heat-shock protein that was retained in the ER lumen due to a specific "KDEL" sequence at its C terminus (1).

After a brief detour into receptor biology as a postdoc with Tom Maniatis, Munro began his own lab and as an independent group leader moved from studying the ER to the Golgi. He's demonstrated that peripheral Golgi proteins are targeted to the organelle through a combination of lipid and small GTPase binding (2, 3), whereas Golgi-resident enzymes depend on their transmembrane domains for their localization (4). More recently, Munro confirmed a long-held theory of his that transmembrane domain length correlates with the localization of integral membrane proteins (5), possibly due to variations in lipid composition that alter membrane thickness. He has also proposed that Golgi-resident coiled-coil proteins act as "tentacles" that catch incoming vesicles (6) and has poked holes in the "lipid raft" theory of membrane microdomains (7). In a recent interview, Munro discussed his scientific identity and his targets for the future.

> "My intention wasn't to say that rafts don't exist, but that one has to be cautious."

EARLY IDENTITY

Where did you grow up?

I was born in Cambridge, England, and I've lived here most of my life. My dad, Alan, was a cellular immunologist at Cambridge University and then in biotech before he retired. He was certainly a strong influence on me, but I was always interested in biology. I collected caterpillars and went fishing and bird watching as a boy.

I decided that I didn't want to go into immunology like my dad. But through him I learnt about the molecular biology revolution that was happening, and that all seemed very exciting. I studied Biochemistry at Oxford as an undergraduate. Molecular biology hadn't quite reached Oxford yet, but there was a small amount going on, and I became interested in gene transcription.

How did that interest lead you to join Hugh Pelham's lab for your PhD?

He was working on the transcription of heat-shock genes at that time. But he was trying to diversify a bit when I got there, so he said, "Why don't you work on heat-shock proteins and try to understand what they're actually doing?" He wanted me to study Hsp70, but a competing lab was reluctant to send us antibodies. So Hugh had to devise another way of studying the protein and came up with the idea of epitope tagging. My first paper showed that this approach worked. It took a while to catch on as a technique, but eventually it spread very widely.

I then used epitope tagging to identify and localize new members of the Hsp70 family and found one—BiP/Grp78—that localized to the lumen of the ER and was associated with unassembled immunoglobulin heavy chains. This provided early evidence for a role of heat-shock proteins in protein biogenesis. We also wondered how BiP/Grp78 was retained in the ER as it lacked a transmembrane domain. Luckily, it was the second lumenal ER protein to be cloned and sequenced, and we noticed this conserved KDEL sequence at the C terminus, which I then showed was an ER retention signal.

So that was the beginning of your interest in organelle identity and protein localization…

Exactly. It really introduced me to the field of cell biology, which at the time was only just starting to become molecular. I'm fascinated by the whole question of how a cell is organized and how proteins localize to different parts of the cell, because it's very visually appealing. You can look at cells and see that a protein is not randomly distributed—it's clearly in a specific place. That tells you that the protein has a signal on it, and that there's something that recognizes that signal. It's a particularly fascinating issue for proteins in the endomembrane system, where proteins are constantly moving around. Resident proteins have to remain in place whilst cargo proteins traffic through the system.

IMAGE COURTESY OF MRC CAMBRIDGE

Sean Munro

LOCALIZING TO THE GOLGI

Why focus on the Golgi?

I was looking around for an interesting cell biological problem, and there were two things that inspired me. One was cell movement, so I started to look at neutrophil chemotaxis. During my postdoc with Tom Maniatis I'd developed methods to clone receptors, so I tried to identify the chemotactic receptor. I accidentally cloned some other neutrophil receptors instead, one of which turned out to be related to the brain receptor for cannabinoids. I spent quite a long time proving that this was a second cannabinoid receptor, but I stopped working on it after that first paper. Ironically, that's still my most cited independent paper because of the huge pharmaceutical interest in cannabinoid derivatives, and it's the only paper I've ever published that the media and my non-scientist friends were interested in.

So perhaps I should've stayed with that, but it wasn't clear where it was going to lead at that point. In the meantime, I'd been looking at these Golgi enzymes. They were just beginning to be cloned, and I wondered how they were targeted to the Golgi. No one had

IMAGE COURTESY OF KATJA RÖPER

Munro grapples with the Golgi.

Text and Interview by Ben Short
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done anything on that. We found quite quickly that the transmembrane domain (TMD) was involved in their retention in the Golgi, and I've been working on Golgi protein targeting ever since.

You recently validated an idea you've had for a long time about TMD length and protein targeting…

I noticed that Golgi protein TMDs seemed shorter than those of similar proteins that went to the cell surface. There weren't that many genes cloned at the time, but from the small data set we could collect, the difference appeared to be statistically significant. So Mark Bretscher and I published this idea and discussed how it might relate to changes in lipid composition and bilayer thickness. And then I did some experiments with synthetic TMDs of varying lengths that targeted proteins to different places.

Now, we've gone back and analyzed much bigger data sets, and it's clear that the original differences in length that we observed are absolutely true. We also found differences in the amino acid composition of TMDs in proteins targeted to different organelles, which may contribute to protein sorting as well.

But we've yet to prove that the plasma membrane really is thicker and that this is the mechanism of protein sorting. It's a very tricky thing to do because you can't manipulate lipid composition in cells without inducing many indirect effects. The other complication is that cholesterol and sphingolipids—which can change bilayer thickness—have also been suggested to form lipid rafts at the plasma membrane. That's slightly incompatible with our view in which the plasma membrane is essentially one big raft that's thick and highly ordered.

Is that why you're skeptical of lipid rafts?

Yes. A few years ago I wrote a review that was critical of the field. My intention wasn't to say that rafts don't exist, but that one has to be cautious. I think it was useful because it forced the field to realize that there were problems with some of their methodologies, particularly detergent resistance. I think raft experts appreciated the problem, but a lot of people had picked up this method and thought it provided an unequivocal way of showing that a protein was clustered in microdomains at the cell surface. I think the review encouraged people to be a bit more critical and use better imaging methods to look for protein clustering.

I think the original idea of rafts as relatively large, long-lived platforms sitting in the plasma membrane isn't really tenable anymore. But the idea has evolved a bit—the current model seems to be that rafts are very small and very transient. That may be the case, but I don't think that's been definitively proven either. So I remain skeptical.

> "Golgins… surround the Golgi in a network of tentacles."

FUTURE TARGETS

What about peripheral membrane protein targeting?

That's become the main focus of my lab. We're particularly interested in golgins—coiled-coil proteins that localize to the Golgi—because there are a lot of them, and people have suggested they have various different functions. We identified the GRIP domain, which targets several coiled-coil proteins to the Golgi by binding the GTPase Arl1. We recently found that many golgins also contain multiple binding sites for Rab GTPases. These don't seem to be involved in Golgi targeting, but we think it allows golgins to capture membranes containing different Rabs. We propose that the golgins act together to surround the Golgi in a network of tentacles studded with Rab-binding sites, to capture incoming vesicles or other Golgi cisternae. I actually wanted to call it a furry Golgi, but the reviewers didn't like that!

What are you working on at the moment?

We're working on these Golgi tentacles, trying to show that that's what golgins are really doing. In addition, having found these Rab-binding sites on the golgins, we've become interested in some of the less-well characterized members of the Rab family. So we're doing biochemistry and genetics to look at their function, mostly in *Drosophila*.

Then we're trying to work out how transmembrane domains get sorted by length. One thing I've become interested in is the idea that proteins are at a very high density in biological membranes—much higher than in the textbook cartoon of the odd potato floating around in a sea of lipids. So the proteins themselves may have a profound effect on a bilayer's thickness and other physical properties. What's appealing about this is that maybe you could sort lipids by collecting proteins of certain properties together. So, protein clustering during vesicle formation could explain quite a lot about lipid sorting, which is poorly understood at the moment.

Can you imagine what you might be if you weren't a scientist?

My mum worked as a careers advisor, and she was quite keen that I didn't become a scientist. I think she got a bit fed up waiting for my dad to come home late from the lab every night. So she encouraged me to become a barrister, because I always liked arguing with her. But really I feel lucky that I went into cell biology at a time when the molecular revolution happened. There's been such amazing progress since I first started in science. I'm lucky to have been born at the right time to have taken part in it.

1. Munro, S., and H.R. Pelham. 1987. *Cell.* 48:899–907.
2. Levine, T.P., and S. Munro. 2002. *Curr. Biol.* 12:695–704.
3. Panic, B., et al. 2003. *Curr. Biol.* 13:405–410.
4. Munro, S. 1991. *EMBO J.* 10:3577–3588.
5. Sharpe, H.J., et al. 2010. *Cell.* 142:158–169.
6. Sinka, R., et al. 2008. *J. Cell Biol.* 183:607–615.
7. Munro, S. 2003. *Cell.* 115:377–388.

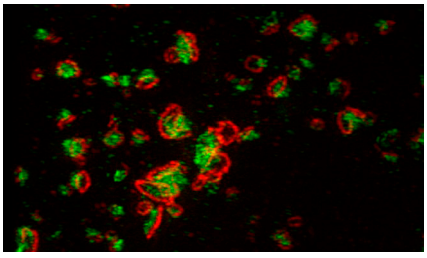

IMAGE COURTESY OF ALISON GILLINGHAM AND SEAN MUNRO

The golgin giantin (red) surrounds Golgi stacks (green).