Matthew Freeman: The sharp end of rhomboids

Freeman studies the functions and roles of rhomboid superfamily proteins.

PHOTO COURTESY OF JUDIE WALDMANN

Matthew Freeman

What do development of the *Drosophila* eye and inflammatory signaling in mice have in common? They both depend upon proteins of the rhomboid superfamily, a widely expressed group of proteins conserved in almost all organisms so far examined, from bacteria to metazoans.

Matthew Freeman first encountered rhomboids while studying EGF receptor signaling (1) in the developing fly eye (2, 3). The discovery that these proteins comprise a novel family of serine proteases (3) galvanized his group to explore the functional consequences of rhomboid activity and that of a group of related but catalytically inactive proteins called iRhoms (4, 5). Rhomboid superfamily proteins have a surprising breadth of expression and diversity of roles that are only now becoming clear, as we learned when Freeman spoke with us from his new office at Oxford University.

EYE ON THE FLY

You did your PhD at Imperial College London…

Yes. That's when I worked on *Drosophila* for the first time. Whilst at Oxford as an undergraduate, I was motivated by the lectures we had on *Drosophila* genetics, so I joined David Glover's lab at Imperial to do my PhD. I was one of his early graduate students and the first one to start working on cell cycle and cell division in flies. We were looking for cell cycle defects amongst early embryos and discovered a rather weird-looking mutation called Gnu, short for "giant nuclei."

And you stayed with the fly for your postdoc…

I've been a fly person throughout my career, although these days my lab doesn't do so much fly stuff. I remember when I was looking for postdocs Gerry Rubin's lab was really the hottest fly lab around at the time, and the fact that it was in Berkeley was another bonus because that sounded like a cool place to live. In Gerry's lab I used the fly eye as a model system to look at developmental biology and questions of cell fate determination.

And that's where you first encountered the rhomboids…

Yes, but I had several other projects as well, and to be honest rhomboids were sort of a backburner project. Even by the time I left Gerry's lab I didn't really see them as my main project because I was working on another gene called *argos*. *argos* and *rhomboid-1* mutants both had interesting phenotypes in the eye, but there was no indication as to what either of them did.

Soon after I got back to England and set up my own lab at the LMB in Cambridge, it became clear that both proteins regulated EGF receptor signaling. That led me into the early work I did demonstrating the significance of the EGF receptor in cell fate decisions in the *Drosophila* eye, and for a long time my main focus was actually on the EGF receptor and other components that regulate EGF receptor signaling. Rhomboids didn't really become a main focus until about seven or eight years after I started my own lab.

> "Early on in your scientific career, it's important to…really think hard about what the important questions are."

NOT A PHILATELIST

What was your lab like in those early years?

The LMB has always had a tradition of very small groups. I think I was the last person to be recruited to the LMB under the old model, where it was seen as slightly pushy and outrageous when I suggested that I would like not only a bench for myself but one for someone else as well. [Laughs] So I had a very small group, and actually it was great. I'm a strong believer that, when one's starting out, it's a real danger to allow your group to get too big too quickly. I think that, early on in your scientific career, it's important to be able to focus and really think hard about what the important questions are. And that's much harder if you're completely new to the game and you're suddenly trying to supervise five or six people without any training in how to do that.

What drew your interest to rhomboids?

The EGF receptor is a very important developmental receptor in flies, and the mammalian homologues are also very important in physiology and disease. It's a canonical tyrosine kinase receptor—an absolute classic—but it was not clear what the normal, physiological regulators of EGF signaling were. So my overall strategy, to the extent that I had one, was to use fly genetics to understand the physiological regulation of EGF receptor signaling.

At first we did a lot of genetic screening, looking for regulators of EGF receptor signaling in flies. We started with *argos*, and then we picked up a gene called *sprouty*, which we did some important work on. But eventually it became clear

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

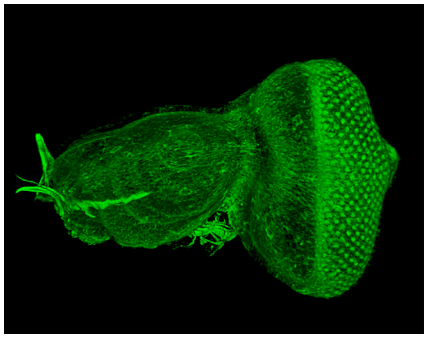

IMAGE COURTESY OF LUIS M. ESCUDERO

A *Drosophila* eye imaginal disc: the favored model for much of Freeman's earlier work.

that Rhomboid-1 was one of the key regulators of *Drosophila* EGF signaling and we really needed to understand rhomboids if we wanted to draw a clear picture of how EGF receptor signaling was regulated. It took us a lot of time and effort to figure out that Rhomboid-1 was a completely novel protease that was responsible for cleaving and activating the ligand that activates the EGF receptor. It was very exciting because it was utterly novel, and it was present in almost all organisms, including bacteria. We had discovered a whole new family of genes.

It soon became clear that this was a very wide family of intramembrane serine proteases, so for some time we were just figuring out what rhomboids did in different contexts. That was entertaining because rhomboids are involved in so many different pathways: they regulate EGF receptor signaling in flies, mitochondrial membrane dynamics in yeast, and surface antigen cleavage in apicocomplexan parasites. But there came a point where I began to worry that if we carried on like that for too long it would become a bit like stamp collecting. There would be fewer and fewer conceptually new things to understand, and that didn't strike me as very exciting. That's when we started getting interested in a class of rhomboid-like molecules, the iRhoms. These look just like rhomboids and are clearly part of the same family, but they don't have catalytic residues. So they aren't active proteases.

We decided to knock out the fly iRhom to see what it does and found that it negatively regulates EGF receptor signaling. At first we assumed that iRhoms were acting in a dominant-negative fashion on rhomboids, but that wasn't right. Instead we discovered that iRhoms degrade the EGF-like ligands of the EGF receptor in flies through a process involving ER-associated degradation.

NEW SUPERFAMILY

But iRhoms have different functions in mammals…

Yes, that's right. Fly people often find it frustrating that, two or three years after they publish something, the mouse story will come out and will get at least as much, if not more, attention because people care a lot more about mammals. So we made a fairly calculated decision that, rather than let someone else have that other glory, we'd look at mice ourselves.

> "We had discovered a whole new family of genes."

To cut a long story short, we found out that mammalian iRhoms are required for the forward trafficking of TNF-α (tumor necrosis factor-α) out of the ER into the later parts of the secretory pathway. It has remained a bit of a mystery why the fly and mouse proteins are doing such different things, but the way we think of it now is that the iRhoms act like adaptor proteins in the ER, which can either interact with the ERAD machinery or with the forward-trafficking machinery to alter trafficking of different client proteins.

Will you go stamp collecting on iRhoms, or will you look more closely at their mechanisms of action?

Both. [Laughs] You're probably aware that I've recently moved to Oxford from Cambridge. My goal is not to allow my lab to get much larger, but it is easier to have a slightly bigger group here than it was in Cambridge. I feel that it's conceptually important to try to understand both what iRhoms do and how they do it. That's especially true now that rhomboids and iRhoms are recognized as being part of a superfamily, most of whose members have completely unknown functions. There are even some very distantly related proteins that are totally uncharacterized. We're very interested in looking at those as well.

How did Oxford lure you away from the LMB?

Skillfully. When they approached me with the idea, I agonized about it for ages. I was incredibly happy in Cambridge. However, I had been there for 20 years, and moving to the Dunn School at Oxford offered the opportunity to work with new colleagues and force myself to see things from a new perspective. And as head of a department I'm given a very free hand to develop it in the way I want.

The final thing was that I had not wanted to leave Cambridge while my kids were in high school because it would have been too disruptive. But they were just reaching college age, and I decided I'd take the plunge and see how the risk plays out. It's been an intense year working to get set up at Oxford, but I think I can see the light at the end of the tunnel now.

1. Freeman, M. 1996. *Cell*. 87:651–660.
2. Freeman, M., B.E. Kimmel, and G.M. Rubin. 1992. *Development*. 116:335–346.
3. Urban, S., J.R. Lee, and M. Freeman. 2001. *Cell*. 107:173–182.
4. Zettl, M., et al. 2011. *Cell*. 145:79–91.
5. Adrain, C., et al. 2012. *Science*. 335:225–228.

The start of the new group in Oxford.