People & Ideas

Marc Freeman: Fishing for the function of fly glia

Freeman uses the power of fly genetics to get to grips with glia.

Approximately 80% of human brain cells are glia, a significant component of the CNS. Yet, until recently, these cells have been regarded almost as mere packaging material for the all-important neurons. Now that view is gradually changing, as Marc Freeman explained in a recent interview.

Freeman's interest in glia began during his postdoc studies at the University of Oregon. There he studied glial cell specification, development, and diversity in the fly (1, 2). Since setting up his own lab at the University of Massachusetts, he has worked on both glial development and the (Wallerian) degeneration of neurons and their phagocytosis by glial cells (3–6).

Recently, Freeman identified a type of glial cell that he believes is the fly equivalent of the mammalian astrocyte. Astrocytes—named for their star-like morphology—are garnering interest amongst researchers as they appear to act not just as neuronal supporters, but as signaling modifiers.

Freeman is excited by where these new stars might take his research. But before discussing his glial research, he was keen to talk about his other important passion.

FLIES AND FLY-FISHING

What made you choose Oregon for your postdoc?

I went there because of Chris Doe. In graduate school, I studied olfaction and behavior in *Drosophila* and learned a huge amount about *Drosophila* genetics, nervous system physiology, and behavior and so on. But I really wanted to study neural development. Chris is one of the leaders in the field, having published on topics like asymmetric cell division, cell fate specification, and patterning of the nervous system. I really liked his work.

When I went for the interview, I had such a great time that I didn't interview anywhere else. He's a wonderful guy, a terrific mentor. I can't say enough good things about him.

How did you like Oregon?

Working in the Doe lab was a treat from beginning to end. Though I had worked on adult fly neurobiology, development proved to be an entirely different world and Chris' lab had people working on seemingly all aspects of nervous system assembly. It was a wonderful group of colleagues and I felt lucky to be there; everybody worked hard (and played hard).

Actually, I'm going back to Oregon with my dad in about three weeks. Oregon is well known for having world-class rivers for fly-fishing, which is something we love to do. We're heading out for about a week and we're just going to disappear into the woods and fish the whole time.

What do you fish for?

Salmon, steelhead, and also trout. Any fish that you can find in a river. Somehow I find rivers to be very peaceful, and a great place to refocus or quietly think. As a postdoc, my favorite was the McKenzie River, a world-class trout fishing river. It comes right down from the Cascade Mountains, and it's absolutely beautiful. Best of all, it was 30 minutes from my lab bench. And the other beautiful thing, once you catch a fish, you can do all kinds of things; you can grill it, or even go ahead and make sushi that night. That's as fresh as it gets.

> "I put up a picture of this fly astrocyte and say, 'What is that from, a mouse or a fly?' And people can't tell the difference."

DISCOVERING A STAR

How does nervous system development compare between fly and mammals?

Motor neurons and interneurons are very similar functionally and developmentally in flies and mammals. So, in general, fly neural development is a fantastic system for comparing with mammals. The field of axon pathfinding is a testament to what flies can contribute to our understanding of nervous system development.

But when it comes to glia, it's less straightforward. In 1995 a fly gene was cloned called glial cells missing (GCM). And everyone got very excited because

Marc Freeman

when you knock out the gene, all the cells that should become glia become neurons. Conversely, if you mis-express it throughout the nervous system, you can transform most neurons into glia. It was therefore suggested to be a master regulator.

People thought, "Okay, let's clone it in mammals." But in mammals GCM doesn't have a major role, if any, in glial cell development. Which was a shocker.

Despite this difference, I was convinced that fly and mammalian glia were very similar. Since GCM could basically force embryonic CNS cells to become glia, we did large-scale analyses comparing GCM overexpressing embryos to wild-type, and collected any gene that looked like it was regulated by GCM, indirectly or directly, and expressed in glia. We reasoned that some of these would be key glial developmental genes. Now we have a list of around 80 to 90 genes. The more genes we looked at, the more it seemed to indicate that while the mechanisms in place to specify glia at early stages are a little different, ultimately the cell type you get out looks very similar at the molecular level.

So the target genes are conserved, rather than the way they are regulated?

Apparently, or at least we can say that's true of many genes expressed late in glial development. Good examples are enzymes that metabolize glutamate for recycling at the synapse—flies and mammalian glia probably use exactly the same mechanism. We also find striking morphological similarities

Text and Interview by Ruth Williams
ruth.williams@rockefeller.edu

between specific subsets of glia. Recently what we've done is take some of the fly genes and make reporters to see where they're expressed in the nervous system. Interestingly, we have found one reporter that labels a really interesting subset of glia that look exactly like mammalian astrocytes.

It had always made me sad when I gave talks to people working on mammalian glia. I would point to fly glia and say, "They're really a lot more like mammalian glia than you think." They weren't necessarily convinced because they looked morphologically distinct. Now, though, I put up a picture of this fly astrocyte and say, "What is that from, a mouse or a fly?" And people can't tell the difference.

GLIA GLEE

What's hot in glial biology right now?

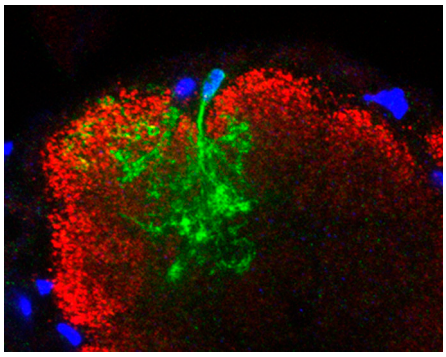
It's become obvious that glia are doing much more than just filling space. For example, they respond to neural activity by dramatically changing their calcium levels. That response can also be transferred from one astrocyte to the next. There's been quite a bit of work trying to figure out how these calcium waves are induced, and what they mean. There's some evidence to suggest that acute changes in calcium levels allow astrocytes to modulate synaptic activity.

A proposed mechanism for glial modulation of synaptic activity would be through the release of gliotransmitters, such as glutamate or other molecules that can modulate synaptic activity. So, that's a point of great interest now—the so-called tripartite synapse: presynaptic neuron, postsynaptic neuron, and glia.

So this is far from just passive packaging?

Yes. The holy grail for the field is to show that glia aren't just support cells, but that they actively participate in information processing. There are continuously emerging data that would argue for it, but we still have a way to go before it becomes a really solid argument in the intact, behaving animal.

Now that we have the fly astrocyte, what we'd love to do is to design an approach where we could look at the tripartite synapse in the fly. For example, we could activate an interneuron that is upstream of a particular motor neuron, and then record from the motor neuron or muscle to which the motor neuron is connected. Then, in that background, you could manipulate glia, and ask, "How does that affect the signaling from interneuron to motor neuron?" We could do that in a living preparation and thus begin to address exactly what is the role of this astrocyte-like cell in synaptic physiology?



Mouse or fly? Freeman has identified the equivalent of astrocytes (green) within the tangle of glial and nerve fibers that make up the *Drosophila* neuropil (red).

Then we could knock out specific genes, such as glutamate transporters, in the astrocytes and see how synaptic physiology changes. In the fly we have all these fancy genetic tricks so these sorts of manipulations are quite simple.

Much of your previous work has been on nerve degeneration and phagocytosis. Are you moving away from that?

I didn't actually plan to study injury in the fly brain, but when people join the lab, I have a list of projects I think would be fun and I let them choose. My very first student chose Wallerian-like degeneration—something like number 23 on the list. That work blossomed, and now we actually have grants to support that and the phagocytosis work. So we are not moving away from that—it's turned out to be too interesting.

But I have just been awarded a Howard Hughes Early Career Scientist award, and that's 100% astrocyte. So we're definitely going to try to bust open the biology of the fly astrocyte. We recently made the tools to go after these questions, and now we have the funding. I think it's going to yield some really exciting results, but we have a long way to go before we're there.

> "We're definitely going to try to bust open the biology of the astrocyte."

Why did you choose UMass?

The chair of the department here is Steve Reppert, a guy who loves invertebrate neurobiology. When I visited UMass towards the end of my postdoc, he was still building the neurobiology department, but I could see that he was on track toward making an outstanding invertebrate neuroscience department. Everyone I met was working on really interesting questions and exploiting the powerful tools available in genetic systems.

Some people say you should really have a balanced department where you have a variety of model organisms—invertebrates, vertebrates, mammals—because then you'll get a more well-rounded input on your projects. But the other way of thinking about it is that if you really focus then you can become extremely good at one particular approach and area of biology. And that's what we have done. If you like invertebrate neurobiology, it's a wonderful place to be.

It seems to be working. You've had some great publications:* Neuron, Nature, *and recently* JCB*! You're on the up-and-up.

We finally reached the pinnacle, right?

Exactly! You've been at UMass for five years. How has it been?

The first two to three years were a lot of really hard work. But now I feel like we're hitting our stride, it's extremely exciting.

Is there anywhere around UMass to go fly fishing?

There's a variety of places you can go. I like Quabbin Reservoir, which is in central Massachusetts. It's actually the reservoir for Boston.

But my favorite is still Oregon. I am looking forward to that trip with my dad.

1. Freeman, M.R., and C.Q. Doe. 2001. *Development.* 128:4103–4112.
2. Freeman, M.R., et al. 2003. *Neuron.* 38:567–580.
3. MacDonald, J.M., et al. 2006. *Neuron.* 50:869–881.
4. Ziegenfuss, J.S., et al. 2008. *Nature.* 453:935–939 Epub 2008 Apr 23.
5. Avery, M.A., et al. 2009. *J. Cell Biol.* 184:501–513.
6. Doherty, J., et al. 2009. *J. Neurosci.* 29:4768–4781.