People & Ideas

Bettina Winckler: Neuronal polarity on her mind

Winckler studies how endocytic processes maintain the polarity of neurons.

Bettina Winckler says she "snuck sideways into neuroscience." She wasn't initially interested in the things that preoccupy most neuroscientists, such as ion channels, synapses, or neuronal networks. Instead, she was fascinated by something that's also present—albeit much less prominently—in other cell types: cellular asymmetry (1).

The quest to understand how neurons maintain their striking morphology (2) has led Winckler down paths more familiar to people who work with fibroblasts than to those who study neurons. Much of her work has focused on the trafficking of the axon-specific membrane protein L1/NgCAM in an effort to understand how it reaches axonal membranes (3). These efforts have led to important insights into how the endocytic pathway in neurons is—and is not—like that in other cell types (4, 5). We asked Winckler to give us a tour through her work from her lab at the University of Virginia.

MAKING THE JUMP

You grew up in Germany, but went to college in the United States?

Yes, I grew up in a town north of Frankfurt. By the time I finished high school, I was pretty sure I wanted to study biology in university. But I didn't want to go to a German university because there was no such thing as a liberal arts education. Basically, when you turned 18 you had to decide if you were going to be a physicist, mathematician, lawyer, or doctor, and I wasn't ready to make that decision.

Fortunately, my parents were very open-minded. They had traveled a lot with my three sisters and I when we were kids, and they encouraged me to go to college for a year in the States. I went to Swarthmore as a biology major, and I loved it there so much that I never came back. I married an American and we live here with our kids, so of course my mom now says that it was a huge mistake to encourage me to go to college here [laughs].

How did you select Frank Solomon's lab for your PhD?

At Swarthmore I did a senior thesis on how asymmetry arises in biology. I was interested in developmental biology and the question of how cell divisions can give rise to dissimilar daughter cells that in turn give rise to hundreds of specialized cell types. I ended up at MIT for graduate school, and at that time first-year students didn't do lab rotations. Instead, every faculty member would give a talk to the first-year class, and we chose who we wanted to work with on the basis of those talks. I think that was a good approach because you were exposed to people and ideas you might not have picked up on by just flipping through the catalog. I really liked Frank's talk because of his work on cellular asymmetry in chicken erythrocytes—I was immediately intrigued.

> "I am working on maybe the craziest asymmetric cell there is: the neuron."

Now I am working on maybe the craziest asymmetric cell there is: the neuron. The average neuron is gigantic compared to most mammalian cells, and it has stunning asymmetry. Issues of location and space are a major challenge for the cell to deal with. That's why I moved to studying neuroscience for my postdoc.

OFF THE BEATEN PATH

How did you end up working on neurons in Ira Mellman's lab—better known at that time for its work on epithelial cells?

I actually did two postdocs. I started out with Mu-Ming Poo when he was at Columbia. That was a very serious neuroscience lab, but when Mu-Ming moved his lab to California, I couldn't go with him because my husband had a job in New York. So, I joined Ira Mellman's lab at Yale.

Bettina Winckler

Ira of course is famous for his work on epithelial cell polarity, but he's very open-minded. He had no problem with me joining his lab to work on neurons, even though nobody before or after me worked on nerve cells in his lab. I began working on a problem I had started thinking about in Mu-Ming's lab, which was: how do neurons maintain polarity? The proteins on dendrites and the cell body are very different from the ones on axons. Once polarity's established, how do you prevent membrane proteins from diffusing into the wrong domain?

So, I started measuring the diffusion of a membrane protein, L1/NgCAM, that's found specifically on the axonal plasma membrane, and I came across something surprising. At least, it was very surprising to me—and probably also to Ira, who wasn't even a neuron person. I found that the first part of the axon, which is called the axon initial segment, has really special diffusional properties. Membrane proteins don't diffuse well there, so it acts like a kind of fence, keeping somatic proteins and axonal proteins separate. We now know that other molecules, such as lipids and cytosolic proteins, also encounter a barrier at the axon initial segment, and this locale in the neuron is critical for neuronal polarity.

L1/NgCAM later turned out to be a useful probe for understanding protein trafficking in neurons…

That came out of being so immersed in Ira's lab, where people think a lot about endosomes. It was already known that

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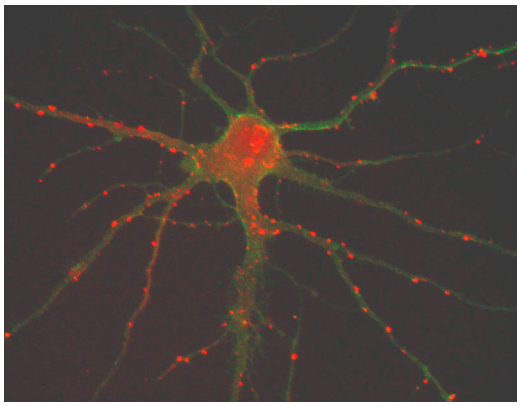


A neuron boasting internalized pools of L1/NgCAM (red).

L1/NgCAM can endocytose. It recycles locally in the growth cone, similarly to the trafficking of adhesion molecules in migrating fibroblasts. But the kind of long-distance polarized recycling that we're working on now was really not something that anybody had thought about very much in neurons. And what we found was surprising: after L1/NgCAM is synthesized in the ER/Golgi, it first traffics to the soma. Then it's immediately endocytosed into endosomes, and only then does L1/NgCAM go to the axon. This kind of trafficking pattern, where a protein appears first at one surface and then uses endocytosis to re-appear at another, is called transcytosis. It's been observed in other cell types, like epithelial cells and hepatocytes.

Initially, many people were skeptical of this pathway that we were describing. But now there are three or four other axonal proteins that also seem to follow a similar pathway, so people are starting to accept it. Now we think that endosomes in neurons act as specialized sorting stations. This is not news to cell biologists, who have known about the powerful sorting capacity of endosomes for a very long time. It just hasn't been looked at in neurons very much.

INTO NEW TERRITORY

To what extent is protein trafficking in neurons analogous to that in other cells?

Carlos Dotti and Kai Simons had a *Cell* paper in 1990, where they were looking at trafficking of viral proteins in neurons compared to epithelial cells. They were the ones who first suggested that the apical domain in epithelia is equivalent to the axonal domain in neurons, while the basolateral domain is equivalent to the soma and dendrites. There are a bunch of proteins for which this is true—they sort according to that pattern—but if you look deeper than that, the analogy starts to break down. For instance, we find that some proteins don't traffic the same way in neurons as they do in fibroblasts, so we can't use fibroblasts as a model for neuronal protein trafficking.

It makes sense to me that you'd have to build a specialized system for neurons because the neuron has spatial requirements—in terms of its exceptional size and shape—that an epithelial cell doesn't have. Neurons also have specialized structures, like synapses, with special rules about how proteins should recycle or not recycle. We think neurons address some of these demands using specialized endosomes and neuronal-specific proteins that do specialized jobs. For instance, we are working on an endosomal compartment that has no equivalent in fibroblasts; it's marked by proteins only expressed in neurons. But we also find examples of the same proteins doing different jobs in different cell types.

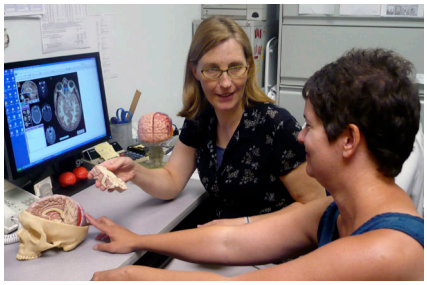
> "I always joke with my colleagues that they're also working on endocytosis; they just don't know it yet."

Besides sending proteins to their proper sites, how might specialized endocytic systems affect neurons?

Endocytosis is a very fast process; cells can remove a huge number of receptors from the cell surface in just a few minutes. In fact, endocytosis and recycling are very important for neurotransmitter receptors and regulating the strength of synapses, for neurotrophic survival signaling, and for regulating axon pathfinding and outgrowth, so it would be helpful to understand how these processes are organized and regulated. It's crucial to uncover the basic workings of cells. Then we can better understand what goes awry in disease or injury, and then hopefully try to repair the damage in intelligent ways. I'm now in the Department of Neuroscience, and I always joke with my colleagues that they're also working on endocytosis and endosomal trafficking; they just don't know it yet [laughs].

For my part, it's only recently that I've started to take seriously the fact that L1/NgCAM is an adhesion molecule —it has ligands—and that probably matters. In the past, we were simply using L1/NgCAM as a probe for the processes we were investigating. But we are now much more interested in the biology of L1/NgCAM and what it does in neurons. Adhesion molecules in neurons promote axon outgrowth, so we're taking a closer look at whether the presence or absence of a ligand affects L1/NgCAM's trafficking. We're also looking at to what degree neurotrophin receptors—which also regulate axon outgrowth—are co-regulated by similar endocytic pathways. We're becoming neuroscientists, I guess!

1. Winckler, B., and F. Solomon. 1991. *Proc. Natl. Acad. Sci. USA.* 88:6033–6037.
2. Winckler, B., et al. 1999. *Nature.* 397:698–701.
3. Wisco, D., et al. 2003. *J. Cell Biol.* 162:1317–1328.
4. Yap, C.C., et al. 2008. *J. Cell Biol.* 180:827–842.
5. Yap, C.C., et al. 2010. *J. Neurosci.* 30:6646–6657.



Winckler and a colleague discussing educational tools in neuroscience.