# Chiara Zurzolo: GPI knows the way to go.

Chiara Zurzolo, Caitlin Sedwick

Published March 30, 2015

# People & Ideas

## Chiara Zurzolo: GPI knows the way to go

Zurzolo studies the trafficking of GPI-anchored proteins and how this links to human disease.

The apical and basolateral membrane domains of polarized epithelial cells contain distinct populations of membrane proteins and lipids that dictate the membrane's functions in epithelial barriers. Accordingly, the targeting of newly synthesized proteins and lipids to these different sites is tightly controlled, with the absence or mistargeting of certain components manifesting in human diseases. The rules governing what goes where, and how this is regulated, are still not completely understood.

Chiara Zurzolo has spent her career working to understand how cell polarity is established, first by investigating the process governing apical sorting of proteins bearing the membrane anchor glycophosphatidylinositol (GPI) (1–3). This effort segued into the study of prion protein, which is GPI linked. Zurzolo's group, currently at the Institut Pasteur in France, has made exciting observations about how prion protein trafficking (4) affects the development of prion disease, and how a newly discovered biological structure may be involved in prion transmission (5). We spoke with her by phone to learn more.

### AN INDEPENDENT STREAK

***What personal qualities define your approach to your work?***

I have always been very independent and determined to do what I want. For example, I demanded to be placed in a research internship when I was in medical school in Naples even though everyone thought I was too young to work in a lab. Then, after only six months of interning in Lucio Nitsch's lab, I learned that the dean of the medical school, Gaetano Salvatore, was arranging summer fellowships at the United States NIH for the school's top students. I went over and introduced myself, "I'm one of your best students, and I want to go to the NIH."

He just laughed at first, but somehow I convinced him because he got on the telephone with his collaborator at the NIH, Harold Edelhoch, and explained that he had a student who was very eager to come over and work. I couldn't believe it. I went home that night and said, "Mamma, I'm leaving! I'm going to the States." [*Laughs*]

I spent two summers at the NIH, and it was a very important experience for me.

***You completed a PhD after your medical degree…***

Yes, I started my PhD with Lucio, studying protein secretion in polarized cells. At that time, many labs were using viruses as tools to follow apical and basolateral sorting of proteins in epithelial cell lines. They observed, for example, that virus envelope proteins such as hemagglutinin trafficked to the apical side, whereas vesicular stomatitis virus G protein was basolateral in Madin-Darby canine kidney (MDCK) epithelial cells. But we were using Fischer rat thyroid (FRT) cells, and I noticed that some viruses trafficked with opposite polarity compared to MDCK cells. Unfortunately, this work was going very slowly. I was almost ready to give up when I decided to leave again for the US and go to Enrique Rodriguez-Boulan's lab at Cornell.

> "Some GPI-APs such as the cellular prion protein, PrPC, go basolateral."

Enrique and Michael Lisanti had discovered that the GPI anchor was an apical sorting signal in MDCK cells. Lucio and I had met Enrique at an EMBO meeting and had sent him our FRT cells to determine whether this was a universal signal. I planned to join Enrique in studying trafficking of GPI-anchored proteins (GPI-APs) in those cells, though when I arrived there, Enrique wanted me to use membrane permeabilization to study the apical and basolateral export machinery in MDCK cells. I agreed to try this project, but on the side, without Enrique knowing, I started a parallel project on FRT cells.

PHOTO COURTESY OF INSTITUT PASTEUR

**Chiara Zurzolo**

That work went very fast, and I quickly showed that a protein called DPPIV, which traffics to the apical surface in fully polarized FRT epithelial cells, actually goes to the basolateral side when the cells are not yet polarized and are still setting up their tight junctions and columnar morphology. In polarized cells, DPP is relocated to the apical side via transcytosis. I brought this paper to Enrique already written, and it was published very quickly with only minor revisions.

### THE VALUE OF PERSISTENCE

***You kept working with FRT cells…***

Yes. I found that GPI-APs were mainly basolateral in FRT cells. That caused a big fuss. Enrique didn't want to believe it because there was a very good explanation available for why GPI should be an apical sorting signal; the lipid raft hypothesis proposed that glycosphingolipids congregate in membranes, and it was thought that GPI-APs might segregate into rafts in the Golgi. Then, because sphingolipids went to the apical cell membrane, GPI-APs would traffic there as well.

We found in another paper that, in fact, glycosphingolipids in FRT cells were not enriched on the apical side. That might explain why GPI-APs traffic basolaterally in FRT cells, but actually the matter is more complicated than that. This is something we are still studying.

Published March 30, 2015

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

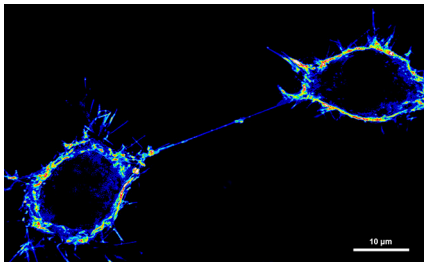


IMAGE COURTESY OF JEAN-YVES TINEVEZ AND ELISE DELAGE

**Actin intensity staining reveals a tunneling nanotube that has formed between two neuronal cells.**

***What, then, determines the trafficking of GPI-APs?***

Later, in my own lab, we started working with MDCK cells where the majority of GPI-APs are apically localized and associated with rafts. But, we found that some GPI-APs such as the cellular prion protein, $PrP^{C}$, go basolateral. Like the apically trafficked GPI-APs, PrP was still associated with lipid rafts; therefore, raft association was not sufficient for apical sorting. However, we saw that apically sorted GPI-APs formed high-molecular-weight complexes in the Golgi, whereas basolaterally sorted ones did not.

We proposed that this mechanism—I call it "oligomerization," but we don't know if there is something else present in these complexes—regulates apical sorting of GPI-APs. Later, we found that oligomerization is sensitive to Golgi cholesterol level and also determines the clustered organization and activity of GPI-APs at the apical surface. But this is not the whole story. We are still studying what else might control GPI-AP clustering and their different behavior in fibroblasts and cancer cells.

## PRIONS GET PASSED ALONG

***What led you to use PrP as a model GPI-AP?***

When I returned to Italy from Cornell, I joined Lucio's lab again as an assistant professor. I now think this was a mistake for my career; you should never return to the lab or town where you started. Also, assistant professors in Italy are not independent. I realized that the only way to be independent was to have my own money, so I immediately applied for grants. One of these was a collaborative effort among many labs to try to understand the mechanism of prion infection. I couldn't study the disease because I didn't have the proper facilities to do that in Italy, so I was just studying PrP's properties as a model GPI-AP. When we discovered it was basolateral, I started to wonder if prion trafficking would be important for its misfolding. If so, then where does this protein misfold?

I wasn't able to study this properly until I got a job offer here at Pasteur. When I could finally work with infected neuronal cells, we demonstrated that the conversion of PrP to the misfolded form, $PrP^{Sc}$, involves the endocytic recycling compartment. Later, we started working on how prion infection is spread and we discovered tunneling nanotubes (TNTs).

> "This could totally change how we think about neurodegenerative diseases."

***How did you come across tunneling nanotubes?***

I had a PhD student who was supposed to work on the role of cholesterol in the folding of PrP. He was a brilliant student, but also one of those people who never do what you tell them to do. I was like that, so I can't criticize, although now that I have my own lab I understand how challenging it is to handle these people. [*Laughs*]

One day he showed me a *Science* paper in which they had found these incredible structures: long, thin tubes of membrane that allow direct communication between the cytoplasm of two cells. There was another paper showing GPI-APs were present on the membranes of these nanotubes, so I knew we had to look at these structures to discover if prion proteins might use TNTs to move between cells.

It was difficult to perfect the imaging conditions needed to find TNTs, but we eventually saw that nanotubes can form between dendritic cells and primary neurons, and that PrP can pass through these tunnels. This has led us to propose that motile dendritic cells may bring prions to neurons, thus allowing the spread of infection from the periphery to and within the brain.

We found that other misfolded amyloid proteins involved in common neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's can also move between neurons through TNTs. We propose that nanotubes may therefore contribute to the progression of neuropathology in these diseases. Our next goal is to show that TNTs exist in tissues. If we can show they're present in the brain, then this could totally change how we think about neurodegenerative diseases and about normal cell-to-cell communication, for example during development.

***In today's funding climate it must be helpful having a direct link to a disease…***

Nowadays you have to have a disease angle to be able to finance your basic science. It's very difficult and a bit sad, actually, and everyone still struggles to get funding. If I could go back, maybe I would dedicate more time to my family and children and less to chasing grants, although I still managed to have a happy family with my two kids and husband. But this is just a dream, as I am addicted to science. Even today, I am often the one who closes the lab in the evening. [*Laughs*]

1. Zurzolo, C., et al. 1993. *J. Cell Biol.* 121:1031–1039.
2. Paladino, S., et al. 2014. *Nat. Chem. Biol.* 10:350–357.
3. Marijanovic, Z., et al. 2009. *PLoS Pathog.* 5:e1000426.
4. Gousset, K., et al. 2009. *Nat. Cell Biol.* 11:328–336.
5. Costanzo, M., and C. Zurzolo. 2013. *Biochem. J.* 452:1–17.

PHOTO COURTESY OF GIORGIO BOWLER

**Zurzolo and family pose for a selfie.**