Tobias Walther: Floating ideas on lipids

Walther studies how cells use and store lipids to maintain membrane composition.

The fluid mosaic model of membrane biology depicts proteins and lipids freely roaming in the lateral plane of the cell's plasma membrane. But in reality, the plasma membrane is highly compartmentalized. One such compartment is delineated by a protein scaffold beneath the membrane called the eisosome (from the Greek word *eis*, meaning "portal," and *soma,* meaning "body").

Tobias Walther, together with his colleague Jason Brickner, carried out the first description and characterization of eisosomes while both were postdocs in Peter Walter's lab at UCSF (1). Subsequently, Walther has worked to better understand eisosomes and the membrane compartment that they define (2–5). But his interests have also evolved to include studies on the homeostatic mechanisms that guide cellular lipid storage and metabolism (6). We spoke to him at his lab at Yale to find out how far he's come in this latest quest.

NOT SO DIFFICULT

When did you first become interested in biology?

I grew up in Bavaria, in Germany. Everyone around me spoke German with a very strong local dialect, and when I got to school I found I couldn't spell anything correctly. I really struggled in school.

The natural sciences became a refuge for me because I didn't have to write so much in those classes. [Laughs] I always achieved good grades in physics, math, chemistry, and biology, but I did badly in German and my other language courses. That's bizarre because I'm actually not bad at learning languages; I speak several languages now. But I almost didn't make it into university because my German was so bad. Fortunately, my school teacher was convinced that, even though I was a marginal student, I was actually a smart kid and that I should really go to university. She made sure that happened.

You published your first paper while you were still an undergrad…

Yes. When I was in university I spent some time as an exchange student at Southern Methodist University in Dallas with a young assistant professor there, Jack Kennell. I worked there for about three months, and at the end of that time we wrote a paper that was published in *Molecular Cell*. I remember thinking, "What is everyone complaining about? Research is really not that hard." [Laughs] And of course it's never again been that easy.

When I returned to Germany I had to write my undergraduate thesis; my paper didn't count for anything. I worked with Jörg Kämper in Regine Kahmann's laboratory for the final part of my thesis. Regine was amazing. She opened a window to the world of research and showed me what's out there, for example by running journal clubs for students, teaching us how to analyze scientific papers, and that kind of thing. That was something no one else was doing at the time.

> " [Eisosomes'] primary function is to segregate specific proteins and membranes into distinct domains."

NOT SO EASY

You seem to have quickly succeeded in your graduate work…

I was in a wonderful group at EMBL headed by Iain Mattaj. But actually for at least my first two years at EMBL, I had nothing. I spent most of my time in cold rooms, either making *Xenopus* egg extracts or trying to purify proteins or antibodies. A lot of things didn't work, and it took forever. Meanwhile, all around me, other people at EMBL were publishing lots of fantastic papers.

PHOTO COURTESY OF DENISE O'BRIEN (AMERICAN DIABETES ASSOCIATION)

Tobias Walther

It took two or three years before I was certain that the assay system I was trying to set up was going to work. But eventually everything came together, and once I had the assay system I was able to address many questions about nuclear pore complex assembly relatively quickly and publish several papers.

What made you choose Peter Walter's lab for your postdoc?

Because it'd been so painful to do all that biochemistry, I wanted to work in a system where I wouldn't have to reinvent the wheel. I also felt that I needed to complement my background in biochemistry with some genetics, and I thought that yeast was the system to go for, because it is easy and fast to do genetics in yeast. But what really connected me to Peter was, first, his personality; he's just great to work with, and that comes across very quickly when you meet him. Second, when I interviewed with him at UCSF, I proposed to him a project that had nothing to do with his work, and he agreed to it right away. That convinced me that this was a lab where I could be independent and develop my own ideas, rather than be a factory worker for someone else's interests.

Text and Interview by Caitlin Sedwick
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What project did you propose to Peter?
It had to do with mitochondrial dynamics, but I actually never ended up working on it. When I arrived at Peter's lab, another postdoc, Jason Brickner, had found these two proteins in an assay he was running to identify interaction partners of ER membrane proteins. It wasn't clear what these proteins were doing, and we started talking and decided to look at whether they localized to the ER. Instead, we found them to be extremely highly abundant and localized in these large structures at the plasma membrane, which we later termed "eisosomes." We thought this was a very peculiar pattern, so we decided to try to figure out what they do. That effort's still ongoing.

What exactly is an eisosome?
Eisosomes are large scaffold structures under the plasma membrane that are composed primarily of Pil1 and of a homologous protein called Lsp1. As far as we know, their primary function is to segregate specific proteins and membranes into distinct domains that seem to be involved in signaling and also probably in endocytosis. We still don't know how eisosomes affect endocytosis, but that is something we're studying quite closely.

NOT ONLY THAT

Your interests have broadened since completing your postdoc…
After returning to Germany to start my first lab at the Max Planck Institute of Biochemistry, I picked up a fascination for how systems—and particularly membranes—maintain homeostasis. We're studying two aspects of this question right now: first, how do membranes maintain their protein and lipid composition, and, second, how do cells store lipids?

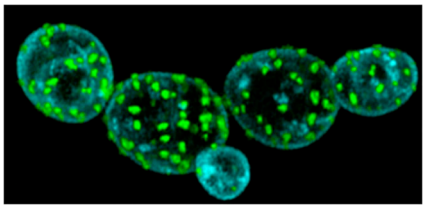


IMAGE COURTESY OF TOBIAS WALTHER

Eisosomes (green) in the budding yeast plasma membrane.

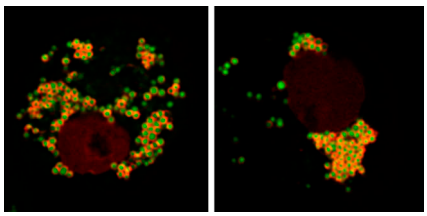
Lipid droplets are a major focus of my lab at this point and the subject of a great ongoing scientific partnership with Bob Farese at UCSF. They're basically balls of fat that are found in all cells. One of their functions is to serve as storage compartments for membrane components. Many of the intermediates for membrane synthesis are toxic, so you don't want them hanging around in the cell. But triglycerol fat is neutral, so the cell can store it as a precursor and, when more membrane is needed, for example during cell division, these stores are utilized.

The other purpose of lipid droplets is to serve as energy stores because triglycerides are a very efficient way to store energy. Not surprisingly, their size depends on the metabolic state of the cell or tissue. For example, if there's excess energy around, a fibroblast will have large lipid droplets. Otherwise, it may not.

> "I picked up a fascination for how systems—and particularly membranes—maintain homeostasis."

One interesting question is why cells sometimes host several small lipid droplets instead of one large one. Lipid droplets are the only place in the cell where you have an obvious phase separation between an organic-phase hydrophobic core and an aqueous cytoplasm. As a consequence, you don't need to explain why they fuse but, instead, why they don't.

Recently, we found that high levels of phosphatidylcholine are needed in order to prevent all the lipid droplets in a cell from coalescing into one large glob. There's a homeostatic mechanism that regulates the synthesis of phosphatidylcholine by targeting the rate-limiting enzyme of that reaction to the surface of lipid droplets, where it detects that there's more phospholipid required. When this mechanism is impaired, the cells' droplets fuse together, and the resulting large lipid droplets become resistant to lipolysis (they can't be easily digested). This is a beautiful example of homeostasis.



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Lipid droplets (green) in *Drosophila* S2 cells are ringed with the rate-limiting enzyme in phosphatidylcholine synthesis (CCT1, red).

What lured you to start a new lab in the United States?
Everyone kept telling me that I was crazy because the funding situation in Europe is excellent. I was at a very good institute in Munich, but my position there would've lasted at most nine years. At some point I would've had to move on, so, when this opportunity came, I took it. I am glad I did because I really like Yale and especially the Department of Cell Biology.

There was also a personal component. My wife is Italian, so, when we're in Germany, I'm responsible for everything, and, when we're in Italy, she is responsible for everything. We both agree that the United States is neutral ground. [Laughs] But now we're starting a family, and that's going to be a real game-changer for us!

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