Julia von Blume: Sorting through the trans-Golgi
von Blume studies the mechanisms of protein sorting in the Golgi.

As a child, Julia von Blume just wasn’t interested in academia. She focused on saving money to buy a horse instead. But after an inspiring high school biology teacher opened her eyes to the beauty of biochemistry, von Blume sold the horse and worked hard to catch up with the others in school so she could study biology at university.

Today, von Blume’s lab at the Max Planck Institute in Martinsried, Germany is investigating how proteins are sorted in the trans-Golgi network (TGN) (1). She first encountered this question as a postdoc in Vivek Malhotra’s lab, where she was confronted with a major puzzle: how the actin-severing protein ADF/cofilin is involved in protein sorting at the TGN (2). The surprising answers to that question (3–5) have since served to raise even more intriguing questions. We called von Blume to learn which of these she’ll be investigating next.

**BIG STEP**

*How did you start working on the role of ADF/cofilin in Golgi protein trafficking?*

During my PhD I studied nuclear transport of protein kinase D with Thomas Seufferlein at the University of Ulm. In 2005, in the middle of my PhD, Vivek Malhotra came to the University and gave a lecture on protein secretion. I was so impressed by his presentation, by his creative work, and by the questions, that I decided I wanted to work on protein transport from the Golgi during my postdoc. I discussed my interests with Vivek during his visit, and he invited me to visit his lab in San Diego, California, just to see how things worked there. So I went there for a week. The group was very close-knit, almost like a family, and very interactive. I really liked that.

After that week, Vivek invited me to do a postdoc in his lab. But when I finished my PhD, I was a bit hesitant to accept his offer. I had just met the man who would later become my husband, and I had a big circle of friends and family here in Germany, so it was a big step for me to move to San Diego. Also, although I really loved science, I didn’t particularly want to be involved in the politics of science. I worried that the politics involved in running my own lab would distract me from research. So I did not immediately say, “Yes, I’ll come.” But Tom Seufferlein strongly encouraged me to pursue this opportunity, and finally he convinced me. Ultimately I was only in San Diego for six months before Vivek accepted to chair a new department at the Center for Genomic Regulation (CRG) in Barcelona. Fortunately the lab move went very well, and I got an EMBO fellowship to continue my work in Vivek’s lab, which was nice.

When I first arrived in his lab, Vivek’s group had just published a genome-wide screen in *Drosophila* S2 cells looking for new regulators of protein secretion. One of the genes they found was *twinstar*, which encodes the *Drosophila* homologue of ADF/cofilin, an actin-severing protein. I was intrigued: how could an actin-severing protein affect protein secretion? Early on I found that several proteins that regulate *twinstar* activity are also required and that this pathway also operated in HeLa cells. What was your initial hypothesis about how ADF/cofilin regulates protein secretion?*

It is known that the actin cytoskeleton is required for budding of transport vesicles at the plasma membrane, so we thought it might be involved in this reaction at the Golgi. But, surprisingly, we did not observe a budding phenotype or an obvious change in Golgi morphology upon knockdown of *twinstar* or its regulators.

I wondered if ADF/cofilin and *twinstar* were really required for the secretion of endogenous proteins and not just the artificial cargo, horseradish peroxidase, that was studied in the original screen. I set up a quantitative mass spectrometry assay to analyze the secretome of ADF/cofilin-deficient cells. At the time, it was very difficult to analyze secretomes because constitutively secreted proteins are not very abundant, but after considerable effort we were able to get this analysis working, and we found that ADF/cofilin is required for the trafficking of a subset of proteins. In the absence of ADF/cofilin, some proteins were not secreted, others were secreted in greater amounts, and another class of proteins was mistargeted. In fact, a resident Golgi protein, Cab45, was secreted under these conditions.

**FROM THE OUTSIDE IN**

*How does ADF/cofilin, a cytosolic protein, affect sorting in the Golgi lumen?*

To find that out, we conducted a screen for cofilin-interacting proteins and identified a protein called secretory pathway calcium ATPase1, or SPCA1. This protein is localized to the TGN and known to pump calcium ions into the Golgi lumen. We thought that ADF/cofilin might somehow regulate the activity of this ATPase.

In collaboration with Miguel Valverde, we designed an assay to measure calcium in the Golgi and found that, in fact, ADF/cofilin and actin were required for calcium uptake. Golgi calcium levels in turn regulate secretion of some soluble cargos from the TGN. We still do not understand how this works, but it was still a big step forward. It was
really remarkable because it’s not known how soluble proteins are sorted in the TGN. The one exception to this is a class of lysosomal hydrolases. These proteins are routed to the lysosome by binding to the mannose 6-phosphorus receptor in the TGN, and the receptor bound to its cargoes is transported out of the TGN by clathrin-coated vesicles. But a cargo receptor has not been identified for other soluble cargo proteins. It seems unlikely there would be specific receptors for each secreted protein…

This was actually one of the questions I wanted to work on when I started my own lab here at the Max Planck Institute, supported by the department chief Reinhard Fässler. But when I came here, I still had a project with Vivek looking at why the Golgi resident protein, Cab45, is secreted from cells when either SPCA1 or ADF/cofilin is lost or when the actin cytoskeleton is perturbed. Cab45 is a calcium-binding protein that is resident in the Golgi. It lacks a membrane anchor but its localization in the lumen of the Golgi is sensitive to calcium. When the Golgi calcium gradient breaks down, Cab45 is secreted by the cells. We reasoned that this protein might actually be a direct component of the sorting pathway. Indeed, knockdown of Cab45 has similar effects on cargo sorting as depletion of ADF/cofilin or SPCA1. We also found that Cab45 binds to other secretory proteins in a calcium-dependent manner. Our hypothesis is that Cab45 is retained in the Golgi by oligomerization and that it forms a matrix that sequesters proteins in specific domains of the TGN.

**Curiouser and Curiouser**

How does ADF/cofilin regulate SPCA1 activity?

We found a 130-amino acid domain in the cytosolic loop 2 of SPCA1 that interacts directly with cofilin. This domain is also required for actin recruitment, and the binding of actin and cofilin to SPCA1 is required for calcium uptake in the TGN.

We’re now investigating how binding of cofilin and actin regulates SPCA1 pump activity. There are three hypotheses we’d like to test. One idea is that cofilin and actin act to cluster SPCA1 within a specific domain or lipid environment of the TGN, and that this could regulate or modulate the activity of the pump. Another possibility is that cofilin and actin may interact with other proteins that are recruited close to the TGN—for example, calcium channels in the ER. Calcium levels in the cytosol are very low and this interaction could recruit the Golgi calcium pump closer to the ER, which has a much higher calcium concentration.

A third possibility is that actin filaments sequester calcium and therefore serve as a source of calcium for the pump. It is known that actin filaments bind magnesium almost equally well as calcium through two binding sites. Interestingly, severing of filaments by cofilin involves the distortion of the filament with the concurrent abrogation of one binding site. It is plausible then that calcium released from the actin filament by cofilin is pumped into the lumen of the TGN by SPCA1.

Where do you see yourself in 10 years?

I hope to have solved the mechanism of calcium-dependent sorting of secretory cargo at the TGN. Besides that, I think I will likely continue to enjoy Wagnerian opera and being a good mom to my daughter, who’s now two years old. In addition, I would like to train young talented scientists to pursue issues of fundamental interest in biology.