Frauke Melchior: How SUMO wrestles other proteins

Melchior's laboratory is exploring the regulation and consequences of protein modification by the small protein SUMO.

Frauke Melchior vividly remembers the afternoon when, as a postdoc in Larry Gerace's laboratory, she developed the film from a particular experiment. It proved that her hypothesis was correct. The mysterious mobility shifts she'd observed in earlier experiments were due to a novel protein modification: addition of the peptide she would name "small ubiquitin-related modifier" (SUMO) (1).

Before that day in the darkroom, Melchior had been searching for a research home. Her graduate work in Marburg, Germany with Helmut Kindl had given her a solid grounding in biochemistry, while a two-year stint as a postdoc with Volker Gerke in Göttingen, Germany had opened doors to molecular cell biology and gotten her interested in nuclear-cytoplasmic transport (2). This interest took her to Gerace's laboratory at the Scripps Research Institute in San Diego, CA, where she helped identify the GTPase Ran as a critical player in nuclear transport (3). It was in the course of these studies that she discovered SUMOylation.

> "It was clear that we'd discovered a new protein modification."

Since then, Melchior has been on a quest to understand how and under what circumstances the SUMO peptide is added to its protein targets (4), and to explore the regulation and functional consequences of this modification (5). Now in Heidelberg (having returned to Germany after her postdoc), she spoke with us about how she got into the ring with SUMO, and her efforts to pin its function to the mat.

BEST LAID PLANS

What first got you interested in science?

My father was a physicist, and my mother had also wanted to become a scientist. She ended up being a chemistry teacher, though, because she had three young children and at that time, science wasn't a realistic option for her. So when I showed an interest in science, my family was very supportive. I decided to study chemistry at University, and eventually stumbled into biochemistry. I didn't really like it at first, but then I went to Helmut Kindl's laboratory to complete my research requirement. I had a lot of fun in his laboratory, and in the end decided to do my master's and PhD thesis there.

I love the puzzle-solving aspect of research—you could have put me on any subject. It just happened to be biochemistry that caught my attention first.

Did you have a plan for what you wanted to do after your PhD?

I'd planned to study plant biochemistry as a postdoc in the US. But then I became pregnant during my PhD. I had always wanted to have children, and in hindsight this turned out to be the best possible timing. But, my significant other, Ludger Hengst, had just started his PhD in Göttingen. I was two years ahead of him in my career, but we wanted to go to the States for our postdocs together. So, I joined Volker Gerke's laboratory in Göttingen as a postdoc for two years to match our timelines together.

Why did you want to go to the States, and San Diego in particular?

Ludger and I considered it a fantastic opportunity to get to know a different culture, and we were looking for a place where there was a really high density of research laboratories so that we could both find our perfect postdoc positions. In Volker's laboratory, I had worked on a project to identify annexins in fission yeast. It turns out that there aren't any but I encountered a protein in my screens, called RNA1, that interested me. Its function was unclear, but there were links in the literature to RNA processing and RNA nuclear export, so I started to read about nuclear-cytoplasmic transport. It was a really young field at that time—not a lot of work had been done at the molecular level. But Larry Gerace's laboratory at Scripps had just published a paper on a new in vitro transport assay. I decided to go there, and it turned out to be the best choice I could have made. Larry was a very supportive and generous advisor.

PHOTO BY MICHAEL MIETHE, BERLIN

Frauke Melchior

BY COINCIDENCE

How did you wind up working on Ran?

Larry's laboratory had made the initial observation that GTPγS inhibited transport in their assay. On Larry's suggestion, I wrote my postdoctoral fellowship on this and followed up this result. I was able to identify that the GTPase Ran was involved in transport. Coincidentally, Ludger had written his postdoctoral fellowship application on analyzing the role of Ran in cell cycle control in yeast. He ended up working on p27 instead, but if you combine the postdoctoral fellowship applications that we sent to the German Research Society, we had it all in there—just not in the same proposal!

How did these studies lead you to find SUMO?

The next logical step after finding the GTPase was to ask where and how GTP hydrolysis takes place. In an amazing

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

coincidence, it turns out that RNA1, which I had first encountered in Volker's laboratory, was actually the RanGAP. So I went back to the RanGAP, but this time in mammalian cells. We were the first ones to make good antibodies to RanGAP, and that allowed us to discover that there are two forms in mammalian cells: one that migrated at the expected size on a gel and another one that migrated 20 kD larger. We discovered that this larger band contained the published sequence of RanGAP, plus a few novel peptides. The only thing similar to these peptides in a BLAST search was ubiquitin. The similarity was only 18%—something that you'd normally consider garbage—but we couldn't find any other matches. We wondered if this might be a modification like ubiquitin. We had recombinant RanGAP, so I did probably the luckiest experiment in my life: we mixed it with HeLa cell extract and ATP and ran a Western blot to see whether the recombinant RanGAP would change its size. That was a moment that I will never forget, when the film came out of the developer and I saw that this crazy idea was correct: we'd converted the 70-kD form to the 90-kD form. It was clear that we'd discovered a new protein modification.

Melchior (second from left) and colleagues are led to new places by SUMO—in this case, up a mountain in Banff, Canada.

Why do you say that was a lucky experiment?

Others had found the SUMO gene and protein before us, but the only people who had the chance to discover that it's a modifier were basically the people who happened to work on RanGAP. SUMO modification is removed by isopeptidases and is very unstable on most proteins. RanGAP is the one protein that remains modified by SUMO when you lyse cells in the absence of isopeptidase inhibitors. I don't know how many laboratories saw some strong, inexplicable band in a gel, but when they tried to catch these bands they were gone. Two other laboratories discovered SUMOylation around the same time as us, and they were both working on RanGAP, too.

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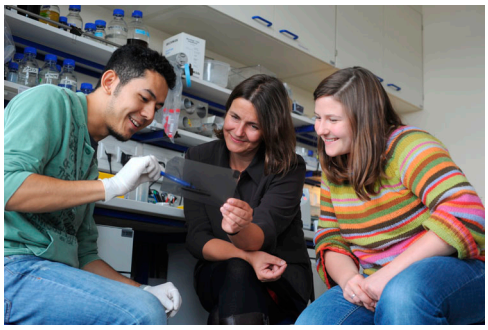
SUMO MUSE

What roles does SUMO perform in cells?

The best analogy would be phosphorylation. Phosphorylation can either help to create a binding module that can be recognized by specialized protein domains, or it can block an interaction surface. In the same way, SUMO can add binding interfaces or remove them. There's also an example where SUMOylation leads to a conformational change of the modified protein. For a long time people assumed that SUMO protects proteins from ubiquitination, but that has turned out not to be true. It's really hard to predict what SUMO modification will do to a given protein without looking at every possibility.



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Melchior and students discuss a new result.

Where are you headed with SUMO now?

We are still very interested in basic mechanisms. We are spending a lot of time characterizing a SUMO E3 ligase called RanBP2, which is a nuclear pore complex protein that binds to SUMOylated RanGAP. This is a fascinating project because it has linked SUMOylation to the nuclear pore, to nuclear transport and the RanGTPase cycle. But I'm convinced that there are more enzymes that regulate SUMOylation—either positively by being E3 ligases or negatively by being isopeptidases—that have yet to be identified. So we are looking for additional enzymes. Related to that, we have another project that personally I find very interesting, on the regulation of SUMOylation by reactive oxygen species. All kinds of stresses—like heat shock, or oxidative stress—lead to global changes in SUMOylation. We think this is because SUMOylation might serve to integrate and coordinate many different processes simultaneously in response to stress. This might be coordinated at the level of SUMO regulatory enzymes.

One of the great things about working on SUMO is that it takes you into all corners of the cell. This makes going to meetings a lot of fun, and also makes it easy to give postdocs a project that they can develop into their own independent research direction.

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