People & Ideas

Stefan Westermann: A close look at kinetochore function

Stefan Westermann is probing the structure and function of the yeast kinetochore.

The faithful separation of genetic material during cell division is accomplished by attaching condensed chromosomes to spindle microtubules and then using forces exerted on the spindle to drag the chromosomes apart into daughter cells. The centromere has a large complex of proteins associated with it called the kinetochore complex, which connects the chromosome to microtubules. Because microtubules are dynamic—constantly growing and shrinking—it's a mystery how the kinetochore attaches chromosomes to spindle fibers.

"When I started out on this project, I somewhat naively thought, 'Oh, this can't be that difficult.'"

Stefan Westermann has been working on the microtubule cytoskeleton for his entire career. He first investigated the tubulin cytoskeleton as a graduate student with Klaus Weber (1, 2). His postdoctoral work with David Drubin and Georjana Barnes probed the kinetochore complex (3) and revealed how the yeast kinetochore is connected to microtubules by a complex called Dam1 (4, 5): Dam1 forms a ring or collar around a microtubule that can move laterally along the length of the tubulin polymer. Westermann is currently heading his own lab at the Research Institute of Molecular Pathology in Vienna, and we caught up with him one evening to talk about his work.

GOOD CHEMISTRY

What got you interested in science?

Well, when I was a child I had one of those home chemistry sets, which was perhaps my first exposure to science. But I would say that I first got really interested in science in "gymnasium" (in Germany, this is the step before university), where I focused on chemistry and English. When it came to making the decision what to study at the university, I thought for a while about pursuing English literature, but then I considered that the job prospects in that field are not so good, so I might be better off focusing on something more practical. I also felt that straight chemistry was a bit dry, so I started studying biochemistry at the University of Hannover.

What made you choose Dr. Klaus Weber's lab at the Max Planck for your Ph.D.?

That happened somewhat by accident. As an undergraduate, I did two laboratory rotations with people who happened to be former students of Klaus's, and they got me thinking that Klaus might be a good person with whom to do a doctoral degree. Klaus is one of the pioneers of the cytoskeleton field, and I was able to take a very classical biochemistry approach to studying the cytoskeleton in his lab.

I was trying to identify proteins involved in tubulin glutamylation (a post-translational modification important for some of tubulin's specialized functions), and to do these studies I was working with this rather unusual organism, the trypanosome *Crithidia fasciculata*. We were using *Crithidia* because it had a high level of glutamylated tubulin, and this made it a good candidate for seeking out the enzymes responsible for this modification.

When I started out on this project, I somewhat naively thought, "Oh, this can't be that difficult." But actually it turned out to be extremely challenging because *Crithidia* is not a common model organism, and it is not worked on by many people. So a lot of the tools that you have with other organisms—like the genome sequences, antibodies, and biochemical tools, etc.—are almost totally absent. I had to do pretty much everything from scratch; when we found a protein we thought was involved with this glutamylating activity, I had to use degenerate PCR primers to try to clone out the cDNA, and that was rather difficult.

Stefan Westermann

We characterized the enzymatic activity and eventually even managed to clone a protein involved in glutamylation—although it turns out the protein we cloned is probably not the enzyme that is doing the glutamylation but rather a cofactor or regulatory protein. Of course, since then other people have made some progress in identifying the actual glutamylating enzyme. But for me, it was a lot of hard work. Everything I have done since then has seemed easy by comparison.

ENGAGED WITH RINGS

How did you arrive at working on the Dam1 complex in your postdoctoral work in David Drubin and Georjana Barnes's lab?

I saw David Drubin at a conference where he spoke about the yeast system and what his lab—he has a joint lab at Berkeley with his wife, Georjana Barnes—was doing to study the cytoskeleton in yeast. I thought it would be great to work in yeast because it is well-studied and there are a huge variety of tools and approaches you can take to working on this organism.

Dam1 was especially interesting because it binds to microtubules, and we knew it had a role at the kinetochore. Our interest was in the question of how the kinetochore attaches to the plus ends of microtubules. Microtubules are constantly growing by adding on tubulin subunits at their plus ends, and then contracting

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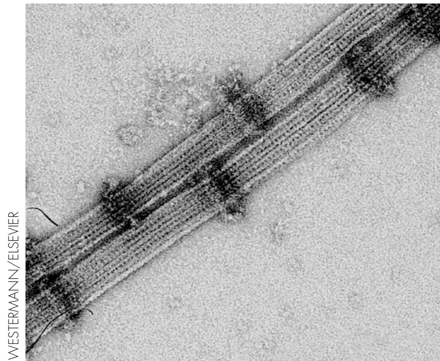


Dam1 rings decorate microtubules.

by catastrophe. So it has been something of a challenge to envision how chromosomes could be attached to such a dynamic structure.

There was some theoretical work dating back to the mid 80s that suggested that the kinetochore might be attached to spindle microtubules via a collar or a cuff-like structure, which could slide up and down along the length of the microtubule. This was a great theory, but the problem was that there was no protein or molecule known which would have the necessary properties to work like this.

We had a collaboration going with Steve Harrison's lab at Harvard Medical School, and we had been sending him some of our baculovirus constructs of Dam1 complex proteins so he could try to express these proteins in cells. This was actually not working out so well, but one of his graduate students, J.J. Miranda, figured out how to make a construct that expressed all ten of the Dam1 complex proteins in bacteria at one time.

This was a breakthrough for us, because when we expressed this construct and looked at the product in the presence of microtubules, we saw that these ten proteins assembled in a ring around the microtubule. The complex expressed very well and was very stable; you could purify it, leave it on the benchtop, and it would be fine.

Also, luckily for us, Eva Nogales—one of the luminaries in the tubulin field—was in the same department at Berkeley; she helped us out with electron microscopy. When we looked at the EM images and saw the Dam1 complex assembled in a ring around microtubules, well, this was very exciting.

Did finding that Dam1 forms a ring confirm earlier theories about kinetochore attachment to spindle microtubules?

At least this was the first protein complex that had the properties that are necessary to function as a kinetochore coupler in vivo. These theories gave us some ideas on how Dam1 might work to attach the centromere to microtubules, but we had some more work to do to show that the formation of the Dam1 ring on microtubules is functionally significant. For example, one of the interesting experiments we did was to show that the Dam1 rings can travel laterally on microtubules.

It's an open question whether the proteins that attach kinetochores to microtubule bundles in higher organisms work in the same manner as they do in yeast. I assume, however, that the mechanical concept with which the Dam1 ring works (the oligomerization of proteins into a ring that then embraces a dynamic polymer) will be a conserved principle and may be relevant in many cellular contexts.

MOVING PAST THEORY

Why did you decide to return to Europe to start your lab?

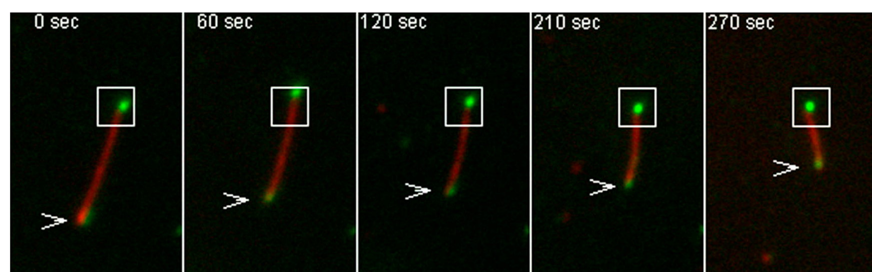
I also interviewed in the States and got some nice offers there, but at the IMP, I had access to greater resources in setting up my laboratory, and there are fewer distractions to research than what you might encounter as an assistant professor in the States. I think the atmosphere here is very supportive of an investigator just starting their own lab. Ultimately, I chose to come to the IMP because they have a good reputation in my field and the facilities are excellent. There is a very good mass spectrometry core facility here, for example. The graduate program also attracts very good students, who I think are the equals of students in places like Berkeley.

"When we saw the Dam1 complex assembled in a ring around microtubules, well, this was very exciting."

What are you focusing on now in your own lab?

We are interested now in following up on the studies with the Dam1 complex. There are many other unanswered questions to address, such as, How is assembly of the Dam1 complex regulated, How does it attach to other kinetochore complexes, and How does it contribute to force generation during mitosis? We are also interested in probing the functions of other kinetochore complexes in yeast and in investigating how other kinetochore components are cooperating with Dam1 to form a functional kinetochore. JCB

1. Westermann, S., et al. 1999. *J. Cell Sci.* 112:2185–2193.
2. Westermann, S., and K. Weber. 2002. *J. Cell Sci.* 115:5003–5012.
3. Westermann, S., et al. 2003. *J. Cell Biol.* 163:215–222.
4. Westermann, S., et al. 2005. *Mol. Cell.* 17:277–290.
5. Westermann, S., et al. 2006. *Nature.* 440:565–569.



A Dam1 ring (green, arrow) slides along the end of a disassembling microtubule (red) anchored to a coverslip (box).