Eva Nogales: See how they run

Nogales studies complex molecular machines using cryoelectron microscopy.

Growing up in Spain during the depressed post–Civil War era, Eva Nogales's parents had to leave school while still very young so they could work to help feed their families. They were determined that their children should have access to the education and the opportunities they had been denied, so Nogales and her brother never lacked anything they needed for school. Nogales also credits her decision to pursue a career in research to three exceptional women: her high school math, biology, and physics teachers, the latter of whom opened her eyes to the elegant logic of physics.

Today, Nogales is renowned for her expertise in cryoelectron microscopy. While some structural biologists take a reductive approach, Nogales feels we can learn important lessons about a molecular machine by viewing as many of its components as possible. Her group at the University of California, Berkeley, and the Lawrence Berkeley National Lab has made many contributions, ranging from foundational discoveries about tubulin and microtubule structure (1, 2) to the operation of kinetochores (3, 4) and transcriptional machinery (5). We called her to discuss how her efforts to visualize multicomponent protein complexes have led to discoveries about their assembly and behavior.

> "Now is a brilliant time to be doing this type of research."

A PRETTY PUZZLE

You were a physics major at university…

That's right. Luckily the university I went to, the Universidad Autónoma de Madrid, was excellent in physics. In Spain you attend the university closest to your address, and most people live with their parents all the way through to their PhD. It just so happened that the Autónoma was about 10 miles from my home town. I enjoyed my studies there, although now that I think about it, I realize that there wasn't a single woman in the physics faculty at my university. In retrospect that seems amazing, but it didn't affect me or discourage me at the time.

The thing that most set me apart was that I really wanted to go abroad—something that was quite rare in Spain. I finally got that opportunity when I met Joan Bordas, the head of the Synchrotron Radiation Source in England, while attending a conference the summer before starting my PhD. He mentioned that the Spanish government had fellowships available for students who wanted to do PhDs in synchrotron radiation research, and also that his group was looking for graduate students. I immediately signed up with him. I impressed him because when he asked how soon I could come, I said, "Well, I'm already packed," because I was attending the conference. "I could go right now."

Was that where you began studying tubulin polymers?

Yes. In my graduate work I was very interested in protein self-assembly. I was, you might say, tasting the flavor of different projects, one of which involved clathrin, and one that concerned tubulin. The prospect that something encoded in the sequence of a protein makes it fold in a certain way and gives it the capacity to organize into higher-order structures posed an interesting problem for a physicist.

It just so happened that when I started looking for postdoctoral positions, my boyfriend—who is now my husband—was offered a position at the new synchrotron being built here in Berkeley, the Advanced Light Source. He said, "Come with me and see whether there's someone there who's interesting to you."

One of the people I met was Ken Downing, who was just starting to work on tubulin. His group wanted to solve the atomic structure of this protein using EM. This was perfect for me because I had studied tubulin in my PhD and had used a little cryoelectron microscopy as a way to complement the studies that we were doing in the synchrotron. I could see the way forward, and working with Ken Downing was what really opened the doors for me to have an independent career.

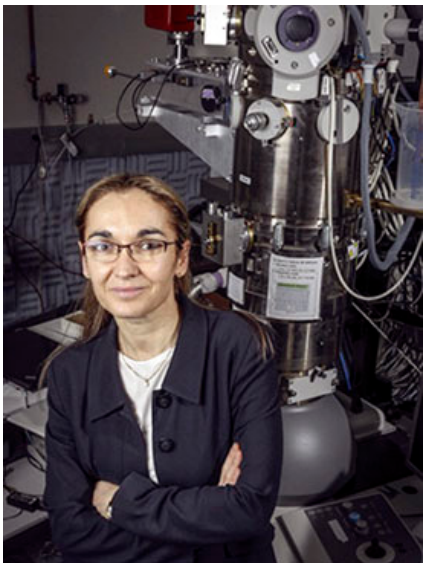

PHOTO COURTESY OF HOWARD HUGHES MEDICAL INSTITUTE

Eva Nogales

LIMITLESS POSSIBILITIES

You were progressively refining the structure of tubulin polymers…

Normally, α/β-tubulin dimers assemble into linear polymers called protofilaments, and protofilaments associate in parallel to form a tube. But during my postdoc we were using zinc to make protofilaments associate in an antiparallel fashion so that, instead of forming a closed tube, they would make a sheet. At the time this was the best that we could do to approach the structure of the microtubule.

For many years we didn't know the details of how protofilaments associate to make a microtubule. It is only now that we are able to get sufficient resolution with the physiological polymer, the microtubule. We've now reached a critical time when the kind of knowledge that we can obtain is truly at the atomic level.

This is now possible partly because of new computational approaches. But there has also been a change in the detector technology used in cryoelectron microscopy, which is revolutionizing both the resolution and speed with which we can obtain structures. The technique is basically exploding right now. Everybody wants to use it. Now is a brilliant time to be doing this type of research because we're less limited by the

Downloaded from http://rupress.org/jcb/article-pdf/209/4/472/1594597/jcb_2094pi.pdf by guest on 05 December 2025

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

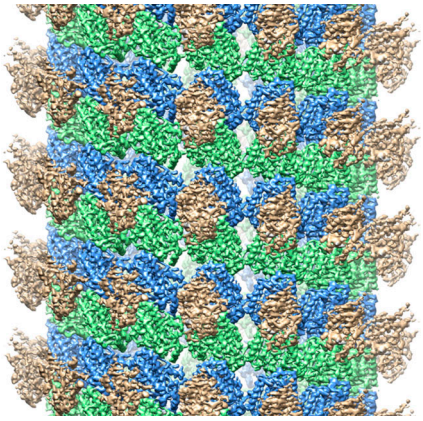

IMAGE COURTESY OF RUI ZHANG AND EVA NOGALES

α-Tubulin (green) and β-tubulin (blue) subunits organize into a microtubule. This one is decorated with kinesin (brown).

instrument and more dependent on biochemistry, the relevance of our questions, and the way we go about pursuing them.

With this expertise in demand, how do you decide what collaborations to pursue?

The main theme that runs through all our projects is that visualization of the molecular machinery that carries out important biological processes allows us to understand biological mechanisms. So, my lab takes on two different types of projects. First are those that we embrace fully, with funding dedicated to them. Sometimes these start off as collaborations. Our work on transcription, for example, started out this way. When I came to Berkeley, my colleague Robert Tjian was studying this complex, TFIID, which is very large and can only be purified in very small amounts. Because of that, there was no structural information on it. Cryoelectron microscopy was an ideal methodology to start visualizing it. But once we started working on that complex, we began adding more components and looking at different aspects. It became a major part of my lab's work.

We also have other types of studies that are truly collaborative, where people come to us looking for our expertise to answer specific questions. These are not at the core of our studies, so we'll take them on only if we think they're feasible, and if we think the system is so biologically relevant and exciting that the structures we produce are going to have a lot of impact. Often the postdocs who work on these projects take them away with them afterwards, whereas the other projects remain themes of my lab.

EUREKA MOMENT

One of these themes has been how nucleotide binding by tubulin affects polymer structure…

The β subunit of the tubulin dimer is the one that hydrolyzes GTP and exchanges nucleotide. What we've seen is that hydrolysis is brought about by contact with the α subunit in the following dimer. As a new dimer is added to the microtubule, it provides amino acids in the α subunit that are required for nucleotide hydrolysis. What's interesting is that hydrolysis doesn't seem to change the structure of the β subunit itself very much, but it has a significant effect on the α subunit above it so that upon hydrolysis the whole dimer lattice compresses and the contacts between tubulin dimers get compacted. That process generates strain in the polymer, but because hydrolysis doesn't occur immediately, microtubules tend to have a so-called GTP cap at their growing end where the tubulin is still bound to GTP and is not strained. This stabilizes the growing filament. But if at any point GTP-binding subunits are not added quickly enough to the end, or if a GDP-bound subunit binds to the end and that GTP cap is lost, you're left with a strained lattice that just breaks apart as the lateral contacts between protofilaments unzip and protofilaments peel away.

> "I actually had one of those eureka moments when I looked at it."

What brought you to study kinetochore proteins?

The dynamic behavior and disassembly of microtubules is critical during mitosis to pull chromosomes apart. We know that kinetochores grab onto microtubules by binding at the end of the microtubule, but also that depolymerizing microtubules peel apart at their ends. So how can it be that kinetochores and chromosomes don't fall off depolymerizing microtubules, but instead use that energy to move with the microtubule end?

This is almost incomprehensible if you don't take into account how microtubules peel and how kinetochores interact with microtubule ends. We started investigating this question through a collaboration with my colleagues at UC Berkeley, Georjana Barnes and David Drubin. They had identified and purified the kinetochore complex Dam1 in yeast and asked me to have a look at it by cryo-EM.

Because this was a brand-new project, I looked at the sample myself. Then I actually had one of those eureka moments when I looked at it and saw that the complex self-assembles into rings around the microtubule. It was a beautiful thing, one of those cases where just the image tells you a lot about how the complex functions: we postulated that these complexes are able to move with the depolymerizing microtubule end by moving in front of the unpeeling strands. Later we extended this work to another kinetochore protein, NDC80, which is conserved from yeast all the way to humans, and now we're working to build the whole kinetochore so we can visualize it with as much detail as possible.

1. Nogales, E., et al. 1998. *Nature*. 391:199–203.
2. Alushin, G.M., et al. 2014. *Cell*. 157:1117–1129.
3. Wang, H.-W., et al. 2007. *Nat. Struct. Mol. Biol.* 14:721–726.
4. Alushin, G.M., et al. 2010. *Nature*. 467:805–810.
5. He, Y., et al. 2013. *Nature*. 495:481–486.

PHOTO COURTESY OF SWEETA MAHANTI

Nogales's children are proud that their parents are making discoveries that are going into textbooks.

Downloaded from http://rupress.org/jcb/article-pdf/209/4/472/1594597/jcb_2094pi.pdf by guest on 05 December 2025