Profile of Eva Nogales

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Capturing the structural intricacies of a biomolecule whose function depends on its flexibility is akin to finding microscopic needles in a moving haystack. Freezing samples at cryogenic temperatures allows biophysicist Eva Nogales the opportunity to observe these molecules at high resolution in a near-native environment. Using cryo-electron microscopy (cryo-EM), Nogales, a structural biologist at the University of California, Berkeley, has watched transcription factors in action, teased apart the structure of microtubules, and revealed details of the machinery for chromosome segregation during mitosis (1–3). In her Inaugural Article, Nogales, elected to the National Academy of Sciences in 2015, uses cryo-EM to visualize, at a near-atomic level, how an essential component of the mitotic spindle maintains critical contact with microtubules to aid cell division (4).

Nogales was born in Colmenar Viejo, north of Madrid, to parents who grew up in post-Civil War Spain. "They had to start working very early—after elementary school," she says. As a result, Nogales explains, "They were obsessed with my brother and I getting an education and white collar jobs. We didn't have much, but my parents made sure we had new books and could concentrate on school."

During high school, Nogales pondered a career in medicine, but was soon turned off by reading medical textbooks. "I found it too gruesome. I went as extreme opposite as I could," she says of her choice to study physics. Nogales credits her interest in science to her biology, math, and physics teachers in high school. "They were truly remarkable women, and I still keep in touch with them." Just after her election to the Academy in 2015, while a visiting scholar at the Spanish National Cancer Research Centre (CNIO), Nogales arranged a reunion with her three former teachers.

Nogales enrolled in Universidad Autónoma de Madrid, which she notes had one of Spain's best physics programs. Nogales graduated with a bachelor's degree in 1988. A chance meeting with the director of the United Kingdom's Synchrotron Radiation Source who was on a recruiting trip inspired Nogales. She recalls telling him, "I want to be one of those. I am ready. I am packed, and I have my luggage."

Important Introductions

Nogales' move to Daresbury Laboratory in England for her doctorate also came with a switch to biology. With advisor Joan Bordas, Nogales worked to visualize the assembly of tubulin, a protein found in the cellular backbone, in the presence of anticancer drugs (5). Tubulin forms microtubules, which are biopolymers that provide critical cellular scaffolding and form the spindles along which chromosomes segregate during cell division. Nogales used small-angle X-ray scattering (SAXS) to visualize polymer assembly. But SAXS could not provide enough information about the structures, so Nogales used cryo-EM to help interpret the data. Cryo-EM uses low temperatures to immobilize and preserve samples and does not require crystallization, allowing molecules to be viewed in their native aqueous environments, a technique that has since become useful for structural biologists.

Eva Nogales. Image courtesy of Mark Joseph Hanson (photographer).

When Nogales graduated with her doctorate in biophysics from Keele University in 1993, it was well before the dawn of the cryo-EM revolution. "At the time, very few people were doing it," Nogales explains. But the switch to biology and the introduction to microtubules and cryo-EM would inform her future work. "It put me exactly on the right path." When Nogales' then-boyfriend and now-husband received a job offer at the Lawrence Berkeley National Laboratory, Nogales met with biophysicist Robert Glaeser, whom she refers to as the "grandfather of cryo-EM." Glaeser suggested she talk with biophysicist Kenneth Downing, who was pursuing the atomic structure of tubulin using electron crystallography.

Like any polymer, tubulin's self-assembling tendency makes it hard to crystallize. "Polymer growth and crystal growth are incompatible," Nogales explains. The researchers used zinc to initiate an aberrant

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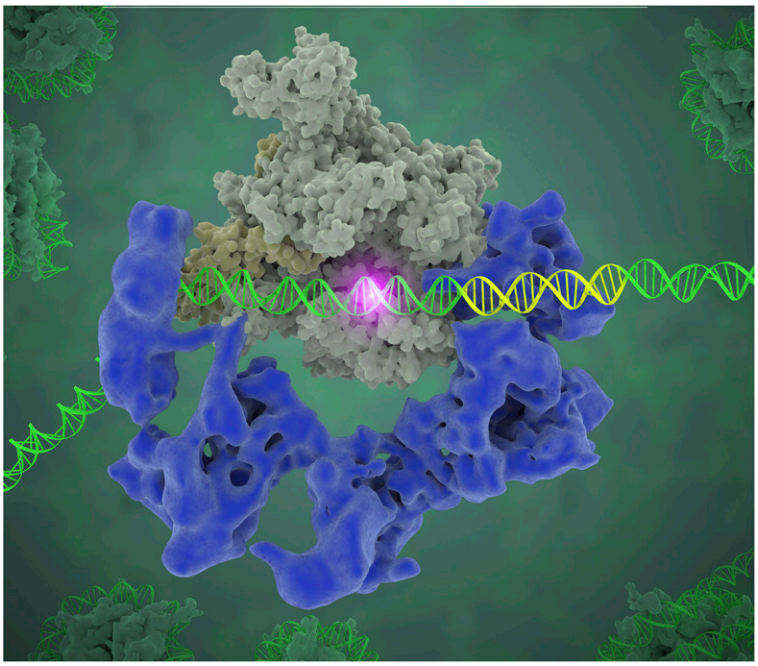

Artistic rendition of the transcriptional machinery bound to DNA at the start of a gene. Illustration courtesy of Janet Iwasa and Robert Louder (artists).

polymerization that gave rise to 2D crystals, and added taxol to stabilize the crystals. "2D crystals were then ideal subjects for cryo-EM structure determination," she says. In Downing's laboratory, Nogales revealed the first structure of the αβ-tubulin dimer at 3.7 Å and described how it interacts longitudinally to make protofilaments (6).

Intractable Subject

After her postdoctoral training ended in 1998, Nogales became an assistant professor at the University of California, Berkeley, and started to use single particle cryo-EM, which does not use crystallization of the proteins. She recalls an early conversation with Robert Tjian, "a world expert in transcription" who thought that single particle cryo-EM would be good for transcription complexes. "That opened up for me a completely new field that is now half of my lab. Transcription initiation complexes suffer from every single problem you can imagine in structural biology. They are almost intractable," says Nogales. "They can only be purified in small amounts, they are not stable, and they are very flexible."

In 1999, Nogales and Tjian solved the low-resolution structure of human transcription factor IID (TFIID), but it took another 17 years to describe its dramatic flexibility and how the flexibility is important for the recognition of DNA sequences (1, 7, 8). The complex needs to be able to switch shape to interact with cofactors, bind DNA, and allow complex regulation. Nogales characterizes the work, showing where the many pieces of TFIID are located with respect to each other and the DNA, as just the beginning. "Now we have to start adding in the regulation," she says, by incorporating a multitude of molecules that affect transcription. She notes that her laboratory has already visualized a preinitiation complex with more than 30 proteins in several functional states (9).

In 2000, Nogales was named a Howard Hughes Medical Institute (HHMI) Investigator. She recalls, "I was a very green assistant professor." The next years would prove stressful: pressures of tenure and HHMI renewal, and sleepless nights with two infants, but still Nogales marveled at how far she had come. "I had no pedigree at any level. My parents weren't in academia. I went to universities only a few know," but, offering inspiring advice, she says, "I had opportunities that came my way. I grabbed them and ran with them."

Rapid Evolution

As Nogales' career matured, so did cryo-EM. Equipment and software packages to collect and analyze the data were constantly improving, but, according to Nogales, "The quantum leap came about three years ago." At the time, she explains, charge-coupled device (CCD) cameras were capturing hundreds or thousands of images, but "the images were suboptimal, especially when taken on the best microscopes with high energy electrons." The new direct electron detectors, hardened to resist damage by the electrons, have much better contrast than CCDs, Nogales says, with the unexpected benefit that the readout is much faster than those of CCDs. In the previous version, she explains, each 2-second exposure produced one image, but the new readouts produce many frames, essentially a 2-second movie.

Nogales notes that this improvement was essential. "When electrons go through our sample, they make the ice move. If you are taking a picture of someone that is moving, the picture will be blurred." The faster readout allowed the alignment of the frames to compensate for that movement. The detector improvements are enabling a much faster output of structures, "a rate that," Nogales notes, "was unbelievable three years ago." As Nogales sees it, the limitations now with cryo-EM are not the scope, the detector, or the software, although all those variables can still be improved, she says. Instead, she says, sample preparation and access to top equipment are limiting.

One of the benefits of cryo-EM is observing the molecule in its natural environment. "We don't put the molecule in the cage of the crystal," Nogales explains. But biomolecules are flexible by design due to their need to conform to different binding partners or carry out work. Thus, in each of the many images, the molecule may be in a different state. "That is hard to deal with computationally," Nogales explains. It does, however, allow her to detect and characterize different states in a sample, which is critical in studying transcription factors.

High Resolutions

Over the years, Nogales has collaborated with a number of Berkeley colleagues, including a long-standing collaboration with biochemist Jennifer Doudna. "We've been working together now for over 14 years. For the last five years, we've been working on CRISPR/Cas9 complexes," says Nogales. Most recently, their studies revealed how thumb-like subunits

of the CRISPR-Cmr complex extend into the target RNA and facilitate its cleavage (10). In 2012, Nogales and biochemist Andreas Martin, whom she had convinced of cryo-EM's applicability to his study of proteosomes, published the structure of more than 30 subunits of the yeast 26S proteasome, revealing the mechanistic underpinnings of ubiquitin-dependent protein degradation (11).

Nogales continues to work on tubulin. In her Inaugural Article (4), she uses cryo-EM to reveal the binding of the protein PRC1 (protein regulating cytokineis-1) to microtubules at the atomic level. "It's a typical example of a study that we can now do at very high resolution." Previous work elsewhere had visualized the full length of the PRC1 molecule at lower resolution as it arranged microtubules bundles in an antiparallel fashion (12), but the details of the PRC1-microtubule interaction and the origin of the 70° cross-bridge angle between microtubules remained unknown. Nogales now shows that it is the orientation of the spectrin domain in PRC1 relative to the microtubule that determines the cross-bridge angle, whereas the rest of PRC1 is highly flexible. The Inaugural Article suggests how this molecular arrangement might allow the capture, orientation, and stabilization of the microtubules in the midspindle during cell division (4). Nogales says she is excited about continuing to investigate the atomic details of the interaction of microtubules with the many factors involved in mitosis.

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