Antonina Roll-Mecak: Decoding the secrets of tubulin complexity

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Antonina Roll-Mecak studies how tubulin diversity modulates the specificity, complexity, and function of the microtubule network.

Antonina Roll-Mecak grew up in Romania during a time when it was still behind the Iron Curtain. Despite the intellectual and political repression of Eastern Europe during the Cold War era, Roll-Mecak benefited from access to the extraordinary state-sponsored science education that offered high-quality training in physics, chemistry, and mathematics. Indeed, her lifelong love of science was fomented at an early age when, while learning about Newtonian mechanics in sixth grade, her father would devise complex pulley-related problems for her to solve. Moving from the Eastern Bloc to New York City, Antonina began her scientific career as an undergraduate studying chemical engineering at the then tuition-free and merit-based Cooper Union for the Advancement of Science and Art. Despite having limited experience in biology, she was accepted into the graduate program at Rockefeller University, where she investigated the mechanisms regulating the GTPases involved in translation initiation with noted structural biologist Stephen Burley before moving on to do her postdoctoral work in the laboratory of Ron Vale at the University of California, San Francisco. While in his lab, she identified a new microtubule severing enzyme.

Roll-Mecak ultimately opened her own laboratory at the National Institutes of Health, where she holds joint appointments at the National Institute of Neurological Disorders and Stroke and the National Heart, Lung, and Blood Institute. Her lab is trying to crack the "tubulin code," examining how genetic and chemical complexity of the tubulin proteins affects the structure and function of microtubules. Here, she discusses some of the formative events and experiences that led her to where she is today.

What is it that drives your interest in examining the tubulin code?

Deceptively uniform ultrastructurally, microtubules are composed of multiple tubulin isoforms that bear a bewildering range of posttranslational modifications, including acetylation, detyrosination, phosphorylation, glutamylation, and glycylation. Tubulin α/β heterodimers consist of a compact folded body and intrinsically disordered C-terminal tails. These tails form a dense lawn on the microtubule surface and serve as binding sites for molecular motors and microtubule-associated proteins. The majority of sequence variation between tubulin isoforms as well as posttranslational modifications concentrates on these intrinsically disordered tails. Some of the modifications we study, such as glutamylation and glycylation, can add amino acid chains that are significantly longer than the tubulin tails themselves. I want to understand how these modifications affect the intrinsic biophysical properties of the microtubule (dynamics and mechanical properties) and how it interacts with cellular effectors. What are the signaling events that lead to these modifications?

"In structural biology, you have nothing until you all of a sudden have everything: a picture of how a protein is put together that nobody else has seen and that you can now use to extract its secrets."

What is your laboratory currently working on?

We want to crack the tubulin code. To crack this code we first need to understand how it is written: the mechanism and regulation of the enzymes that introduce tubulin posttranslational modifications. Second, we need to elucidate how posttranslational modifications and isoform variability affect the basic properties of the microtubule polymer itself. Third, we need to know how spatial and temporal patterns of microtubule modifications are established and propagated. Ultimately, we need to understand how tubulin isoform composition and modifications are interpreted by cellular effectors, regulating their recruitment or activity. My laboratory has developed a biochemical platform to obtain recombinant, isotypically pure human tubulin (1) as well as quantitatively defined, differentially modified tubulins (2) that allow a mechanistic look at the tubulin code. We will continue to try to uncover the basic principles that give rise to the differential specificities of tubulin modification enzymes (3, 4) and how they cooperate and compete to modify the microtubule substrate; to discover how the tubulin code modulates the structure, dynamics, and mechanical properties of microtubules; and to understand how motors and microtubule-associated proteins are influenced by the tubulin code (2) and how their action in turn modulates the behavior of this dynamic polymer. Moreover, we and others have discovered that some of the modifications that were thought to be characteristic of tubulin in fact regulate other molecular players such as histone chaperones and DNA sensors involved in the innate immune response. We have recently shown that glutamylation, the addition of glutamates to tubulin tails, acts as molecular rheostat to control the activity of the microtubule-severing enzyme spastin, suggesting a possible mechanism by which stereotyped subcellular patterns of microtubule glutamylation can regulate local microtubule dynamics through severing. The majority of tubulin in the adult mammalian brain is glutamylated and the number of glutamates on tubulin tails in neurons is distributed in stereotyped patterns: axonal microtubules have long glutamate chains, while the soma and growth cones mostly lack glutamylated microtubules. We anticipate that other microtubule regulators display such graded, spatially controlled responses to glutamylation. In addition, a

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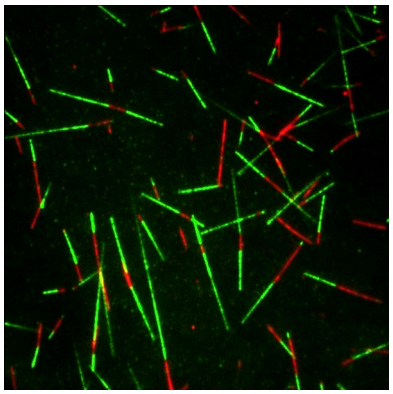

A snapshot of recombinant dynamic single-isoform human α1AβIII microtubules (green) growing from stabilized seeds (red).

recent collaboration with Tiansen Li's laboratory at the Eye Institute has allowed us to show that glutamylation by tubulin tyrosine ligase-like glutamylase TTLL5 is required for the function of a protein essential for normal vision, RPGR, which localizes to the transition zone in photoreceptor cilia (5). It is mutated in retinitis pigmentosa, a degenerative eye disease. Thus, we have become interested in how glutamylation is used by cells to modulate other important pathways.

"Go after a problem you are interested in and don't try to hedge your bets too much. Go all in!"

What kind of approach do you bring to your work?

We use a highly mechanistic and interdisciplinary approach. Like a complex music score that can be broken down into its constituent phrases, so can complex biological phenomena be distilled down and reconstituted in vitro to understand their governing principles that can then serve as the foundations to reconstitute the complex score. We use a variety of techniques in our work from structural biology and classic enzymology to single molecule biophysics, live cell imaging, and modeling.

What did you learn during your PhD and postdoc that helped prepare you for being a group leader?

I had a long dry spell during my PhD and only experienced success at the end of my fourth year. There were small signs along the way that things might work out, but there was also a long history of failure from many other groups on the project. In structural biology, you have nothing until you all of a sudden have everything: a picture of how a protein is put together that nobody else has seen and that you can now use to extract its secrets. While it definitely had its dark moments, my PhD experience gave me the confidence that I can crack problems that remained unsolved for some time. My postdoc with Ron Vale was highly formative. Ron taught me to trust my scientific nose and use any technique at my disposal, either directly or through collaborations, to solve a problem. I also learned how to maintain a competitive environment that was collaborative and friendly.

What were you unprepared for when starting your laboratory?

The paperwork! But I have learned to navigate it efficiently now.

What has been the biggest accomplishment in your career so far?

My biggest accomplishment has been to set up a vibrant laboratory where people challenge themselves and work collaboratively in opening up an exciting realm in microtubule biology where there are so many unsolved basic questions.

What has been the biggest challenge in your career so far?

Deciding when to give up on projects or on people.

Who were your key influences early in your career?

My parents taught me that even the greatest effort seems easy if it is done for something that you love, while my piano teacher taught me focus and discipline. I was a bit of a street urchin in my childhood and my parents were quite concerned about my academic performance until my piano teacher Eniko Orth took me under her wing when I was nine. In addition to the guidance offered by my doctoral and postdoctoral advisors, I benefitted from mentorship by Rod MacKinnon, who introduced me to the experimental craft of protein biochemistry while working side by side with me at the bench when I started graduate school. I was also influenced by Günter Blobel, who impressed on me the importance of in vitro reconstitution in understanding cell biology.

What is the best advice you have been given?

Follow your nose and ignore the prevailing fashions.

What hobbies do you have?

Running a laboratory and having a toddler does not leave much time for hobbies. I enjoy classical music. When I was a graduate student I was able to take in two to three concerts or opera performances a week in between running chromatography columns or sucrose gradients. Going out for performances is a lot harder now with a toddler at home.

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What do you think you would be if you were not a scientist?

I might have been a pianist, but I really have a hard time picturing myself doing anything else other than science. I really love showing up to work in my laboratory.

Any tips for a successful research career?

Go after a problem you are interested in and don't try to hedge your bets too much. Go all in!

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My son turns one! First cake I ever baked.