Gia Voeltz: Shaping ideas about ER shape

Gia Voeltz is using in vitro assays, cell biology, and electron microscopy to investigate how the endoplasmic reticulum (ER) acquires its tubular shape and how this shape supports ER function.

The ER is a membrane-bound organelle that is connected to the nuclear envelope. The ER has a characteristic branching, tubular shape that is conserved across many organisms and cell types (1). How this shape is achieved remains a mystery.

As an undergraduate, Gia Voeltz studied RNA splicing with Manuel Ares (2). This early exposure to research led her to pursue graduate work on RNA stability with Joan Steitz (3). Initially, Voeltz thought she would be studying RNA forever, but as a postdoctoral researcher, she boldly switched to studying how ER shape is created in cells. Using an in vitro assay of tubule formation, she identified the protein family—reticulons—that is responsible for the distinctive tubular ER structure (4, 5).

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Voeltz thinks reticulons create a tubular shape in membranes by occupying more space on the outer leaflet of the organelle than on the inner leaflet. At her new lab at the University of Colorado, Boulder, she is studying this theory and the roles that the tubular ER plays in cell biology. We tracked her down to discuss the intriguing shape her research is taking.

RNA FOREVER

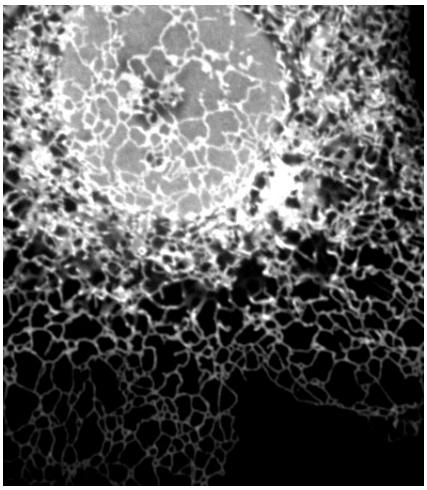
What got you interested in science?

Growing up, I always said, "I'll be anything but a professor," because my parents were both professors in the social sciences, and it was a tough career. I think I was originally interested in being a doctor. It wasn't until I went to college that I got really interested in lab work.

I went to undergrad at Santa Cruz, and that's where I was really formed in a lot of ways. I worked in Manuel Ares's lab on RNA splicing. It was a wonderful place to be an undergrad and get interested in science. At the time, they didn't have a huge graduate program or a lot of postdocs, so the professors depended a lot on undergraduates in the lab. There were high expectations on the work we did. Manny had me working on a pretty independent project, and I really enjoyed having my own project and working directly with him. He served as my first really good mentor and got me interested in research and science.

Was he a major inspiration for you?

Yes, although there have been so many inspirations, including my parents, who introduced me to the life of an academic. But Manny was the one who got me interested in doing research, spent the time to train me early on, and ultimately helped me make some really good decisions. In particular, he told me to go to Yale and work for Joan Steitz. She's an amazing person in the RNA field, really a model scientist and educator, and very much admired.



Voeltz wants to determine how the ER acquires this elaborate tubule structure.

Gia Voeltz

I still did rotations and everything, but I came in hoping that I'd be able to work with Joan. She had so many different projects going on that there was a lot to choose from. I felt like I had been in the RNA field already for a few years, and Santa Cruz was such an RNA university that I guess I thought I'd be in the RNA field forever. I did end up joining Joan's lab to study mRNA stability. When I started graduate school, I didn't know that mRNA stability could be regulated. The first time I ever heard of it was when I went to Joan's lab, and I thought, "That's kind of cool, I'll work on that."

UNEXPLORED TERRAIN

What made you decide to leave the RNA world behind and head to Harvard to work on ER structure?

Part of it was personal. My now husband was already a postdoc at Harvard, so I wanted to go to Boston. As a graduate student, when I was finishing up, I went to almost every seminar by anyone from the Boston area. Tom Rapoport came and gave a seminar in our department, and he talked for about 50 minutes about protein translocation into the ER. But in the last five or ten minutes, he talked about this project he had going on looking at how the ER forms.

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He had an in vitro system for ER formation, and he said, "How does this organelle actually form? Nobody has any idea… If there's anyone out there who'd like to come study it in my lab, they should let me know." It was the same kind of feeling I guess I had when I decided to work on mRNA stability.

"We have a bunch of cool theories about how these proteins might localize and partition in the membrane."

I'd had little exposure to cell biology beyond the classes I took as an undergraduate. In those classes, we learned, "Here's the Golgi, here are the ER and the mitochondria. They look like this, and this is what they do." When I heard Tom's talk, I thought it was a very interesting idea that organelles would be shaped and formed, and that proteins would be involved in changing the shape of a membrane bilayer.

I really studied up on the ER before I went on my postdoc interview with Tom. I read every paper he wrote and other papers, because I felt unprepared for the field. I so overprepared for my postdoc interview that he actually said to me since, "Oh, you did this wonderful job interviewing, you knew every paper I ever wrote." I'm glad I overdid it because then I had a great time working with Tom.

SHAPING A NEW CONCEPT

How did you end up taking the approach you eventually used to look at ER structure?

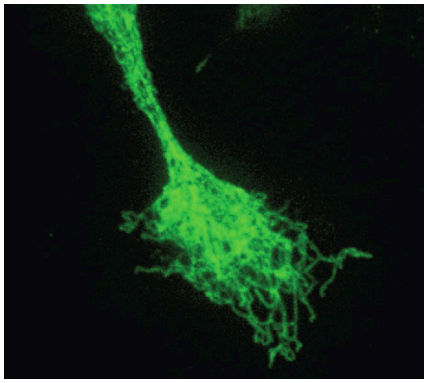
The system was really set up for me. Tom's lab already had this wonderful in vitro system for forming ER tubules from ER vesicles derived from *Xenopus* egg extracts. That was all there and ready to go, and it was a matter of fractionating these membranes and the cytosol and looking for the factors involved.

In retrospect, it seems it should have been obvious that there must be some abundant, integral membrane protein that would maintain this structure. But when I started the project, we started off looking for a soluble cytosolic factor that was important for changing these vesicles into tubules because cytosol was required for the tubules to form in the assay's original conditions.

But it turned out that this was an artifact of the buffer; with the low-salt buffer we were using, the tubules would only form from vesicles if cytosol was around. So we spent some time chasing down this hypothetical cytosolic factor until we realized that if you changed the buffer to a higher salt concentration and then got rid of the cytosol, you still had tubule formation.

What was the hardest challenge you had to overcome in this work?

It was a funny process, how I ended up identifying reticulons. I had noticed the salt effect with the buffer, and I had started reading about aquaporins—water channels



The tubular ER is packed into the growth cone of a neuroblastoma cell.

in the ER—and I thought maybe luminal ER volume might be involved in tubule formation. So I set out to inhibit aquaporins using 5 µM gold, which is a concentration that the literature says inhibits water channels. Sure enough, it inhibited tubule formation.

But it was possible that the gold was just interrupting cysteine bonds in proteins, so I also started trying all sorts of different maleimide reagents, which also react with cysteines. No matter which one I added at 5 µM, whether the compound was huge or small, they all inhibited tubule formation. I was able to pull down modified proteins on beads, and because the concentration of maleimides we needed to inhibit tubule formation was so low, about the only protein it was modifying was reticulon. So that was how we found it.

What have you taken with you to work on in your own lab?

There are some features that reticulons have that favor their localization to tubules. We have a bunch of cool theories about how these proteins might localize and partition in the membrane based on primary structure. My favorite model right now is based on the idea that reticulons have double hairpins that insert into the membrane and generate membrane curvature by occupying more space in the outer membrane leaflet than in the inner leaflet; that could potentially generate membrane curvature.

You can imagine, though, that what it would generate is a sphere rather than a tubule. So it's possible that the protein is oligomerized in a way—for example, in a spiral or helix around ER tubules—that causes it to generate curvature in a defined manner. If that's the case, we'd like to visualize this oligomerization.

That was a huge draw for me to come to Boulder, because here we have this fantastic EM facility and a lot of really good people willing to collaborate with us. I'm also interested in looking for functions of the tubular shape of the ER: how it might affect cellular morphology and whether really unusually shaped cells get that way due in part to the shape of the ER. JCB

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