Junjie Hu: Shape-shifting in the endoplasmic reticulum

Hu investigates proteins that bend and blend ER membranes.

Junjie Hu's doctoral training as a structural biologist in Stevan Hubbard's group at New York University would serve him well. Toward the end of his PhD, a lab head across the hall, David Ron, suggested he apply to Tom Rapoport's lab at Harvard Medical School for a postdoc. The day after he defended his thesis in the fall of 2005, he traveled to Boston to pick up a project on the reticulons and DP1/Yop1p, a class of proteins that shape the ER tubules.

As a postdoc, he showed that either purified Yop1p or the major yeast reticulon, Rtn1p, were sufficient to generate highly curved membrane tubules in vitro (1). The reticulons, however, offered the structural biologist little hope of crystallization, so Hu turned his attention toward the atlastins, dynamin-like GTPases that interact with the tubule-shaping proteins (2).

In the fall of 2008, Hu returned to his home country to set up his own laboratory at the Nankai University in Tianjin, China. In 2013, Hu moved to the Institute of Biophysics, Chinese Academy of Sciences in Beijing.

Returning to his structural roots, his group characterized two conformations of atlastin dimers that illustrated their likely role in homotypic ER fusion events (3). Recently, his laboratory showed that another structural feature of atlastins, the C-terminal amphipathic helix, was also required for efficient fusion (4) and that GTP hydrolysis has a somewhat surprising role in forming fusogenic atlastin dimers (5). Hu spoke with *JCB* about his passion for unraveling the steps of membrane fusion and tips for envisioning proteins in 3D.

TOTALLY TUBULAR

What do you like about the ER?

The ER is a very good model for studying shape versus function because it's a very large and continuous membrane. It's intriguing how just one membrane system can generate all these different shapes with different membrane dynamics, carrying out so many different functions.

In mammalian cells, the ER network is kind of everywhere. It starts with the nuclear envelope, then most of the perinuclear region is sheet-like ER, and then further out is the peripheral reticular network of tubules.

On the other hand, in yeast and plant cells the ER is cortical, right underneath the plasma membrane. There are probably only a few tubules that connect this cortical ER to the nuclear envelope. That's quite intriguing. How is this different organization regulated in different cell types?

What prompted a closer look at atlastins and their role in the ER?

Our collaborator Craig Blackstone, a neurobiologist, has been studying hereditary spastic paraplegia (HSP), in which the gene encoding atlastin is mutated, for a while. In 2008, his group discovered that a dominant-negative form of atlastin changes the shape of the ER—it becomes less branched.

> "It's intriguing how just one membrane system can generate all these different shapes."

We saw their images and it was very shocking because the ER looks just like it does upon reticulon overexpression, with a whole bunch of tubules that don't connect to each other anymore. So we knew this atlastin GTPase must have something to do with tubular ER.

And that something turned out to be ER membrane homotypic fusion?

Yes. Atlastin is part of the dynamin GTPase superfamily. One of the other family members is called mitofusin, a protein that mediates fusion of the mitochondrial outer membrane. The domain structure and topology of mitofusin and atlastin are very similar.

We think the three-way junctions between ER tubules are made by atlastin regulating ER fusion. In yeast, the Sey1p protein, which is the functional homologue of atlastin, localizes as punctae at the three-way junctions.

PHOTO COURTESY OF THE HU LAB

Junjie Hu

LIKE MELDS WITH LIKE

How do mutations in a possible ER fusion protein cause HSP?

This particular disease affects the axon of the cortical spinal motor neuron, which is very long and projects to the lower limbs. We think the connectivity of the ER in these cells is particularly sensitive to mutations in atlastin because the ER has to be continuous to maintain the axon. If there are ER fusion defects, it will break up the ER in the axon and then everything else falls apart.

ER integrity turns out to be important in any cell with long protrusions. Plants don't have atlastins, but they do have an ER-membrane GTPase called RHD3. Mutations in this GTPase cause short and wavy root hairs—the root hair cannot grow as long as it normally does.

Our current view is that ER fusion is essential to all cells, but there are probably back-up, redundant atlastin family members that can compensate in cells without long protrusions.

So the next step was to try to crystallize atlastin?

Yes, I think that's the right way to tackle these questions because if you want to understand the molecular mechanism of these fusion events, the obvious thing to do is to find the structure of the GTPase protein in the different nucleotide-bound states.

Text and Interview by Kendall Powell
kendall2@nasw.org

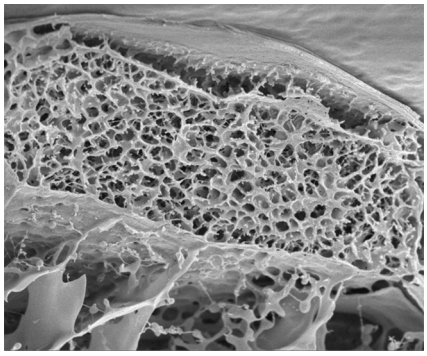

IMAGE COURTESY OF MIAO ZHANG

A scanning electron micrograph of the ER from an *Arabidopsis* leaf cell.

You got crystals using the N-terminal GTPase domain plus three-helix bundle portion of atlastin. What did those structures reveal?

We got the crystal that we call "post-fusion" first, where we think the molecules sit in the same membrane because the three-helix bundle domains are pointing in the same direction, side by side. The bundles actually cross over, like a pair of scissors.

We were already very excited about the first structure because it made so much sense that fusion should be that way.

But I think the second structure was actually more exciting. In this case, the dimer was oriented with the three-helix bundles facing away from each other, as if in opposing membranes.

But the most exciting moment of this study was when we did the trypsin protection assay, where we see that the trypsin digests the dimers differently when you add different GTP analogues. That tells us for sure that these conformations are actually real—that they represent different stages during the GTPase cycle.

NEW FUSION MENU

What does the C-terminal end of atlastin do?

In collaboration with Tom Rapoport's lab, the first thing we did was to make truncations, and we found that we could not lose the C-terminal tail. If you lose the tail, the efficiency of in vitro fusion decreases a lot. There is also a mutation in HSP where the protein loses its tail.

One of the main characteristics of the tail is an amphipathic helix. We did a sequence alignment of all the atlastin family members and we realized that the conservation only ends after this helix, which suggests that the helix is important.

We synthesized a peptide of that helix, just 29 amino acids long, and showed that this peptide could rescue the tailless protein in the in vitro fusion assay.

What role might an amphipathic helix play in fusion?

One way to think of this is as a way of separating lipids so that the hydrophobic part of the lipids is exposed and ready for mixing. If the bilayers are tightly sealed, then it's very difficult for the lipids from two membranes to mix with each other, which is the goal of membrane fusion. We think that atlastin's C-terminal tail disrupts that tight seal.

> "ER integrity turns out to be important in any cell with long protrusions."

What's your current view on how atlastin mediates ER membrane fusion?

The last study we published together with Tom's group reconciles the structural information with findings from the in vitro assays. Initially, we found that when you add a nonhydrolysable form of GTP, GTPγS, you cannot achieve membrane tethering in our assay. That didn't seem to make any sense, because our structure-based model said that dimers form when atlastin binds GTP. Dimer formation between atlastins from two different membranes should pull the two membranes together, so, when you add GTPγS, you would expect to see membrane tethering.

But the reality was that we could only see membrane tethering with the addition of hydrolysable GTP. When we added GTPγS, almost all of the atlastin dimers were cis dimers, sitting in the same membrane. And the cis dimers are so stable that they will no longer dissociate and try to form a trans dimer with a molecule on an opposing membrane. But, if GTP was added, the atlastin dimer hydrolyzes it and becomes a monomer again. So it's only when you have this GTP cycle going on that, at some point, the atlastin has the chance to form trans dimers, which facilitate fusion.

So the cis dimers are a brake, keeping homotypic fusion in check?

Yes, that is one possibility. Maybe you can call this inefficient but maybe the ER doesn't need that much fusion happening. Perhaps it's a way to prevent everything in the ER from fusing together into one big blob.

Also, we used to think that GTP hydrolysis induced the power stroke that drives membrane fusion. But now, we think that most of the GTP is used to make atlastin monomers available for the formation of trans dimers.

Any tips for students who are trying to think about proteins, membranes, and organelles in 3D?

Students should take an intro course to structural biology. They don't have to know how to purify proteins or make crystals, but there are online software tools where students can download a Protein Data Bank file of a protein structure. You can open these and immediately see the protein in 3D, rotate things around, zoom in and zoom out. That kind of practice may help you think of a molecule in a 3D way.

1. Hu, J., et al. 2008. *Science*. 319:1247–1250.
2. Hu, J., et al. 2009. *Cell*. 138:549–561.
3. Bian, X., et al. 2011. *Proc. Natl. Acad. Sci. USA*. 108:3976–3981.
4. Liu, T.Y., et al. 2012. *Proc. Natl. Acad. Sci. USA*. 109:E2146–E2154.
5. Liu, T.Y., et al. 2015. *Proc. Natl. Acad. Sci. USA*. 112:E1851–E1860.

PHOTO COURTESY OF JUNJIE HU

A Hu lab artistic brainstorm of how graduate students envision the ER.