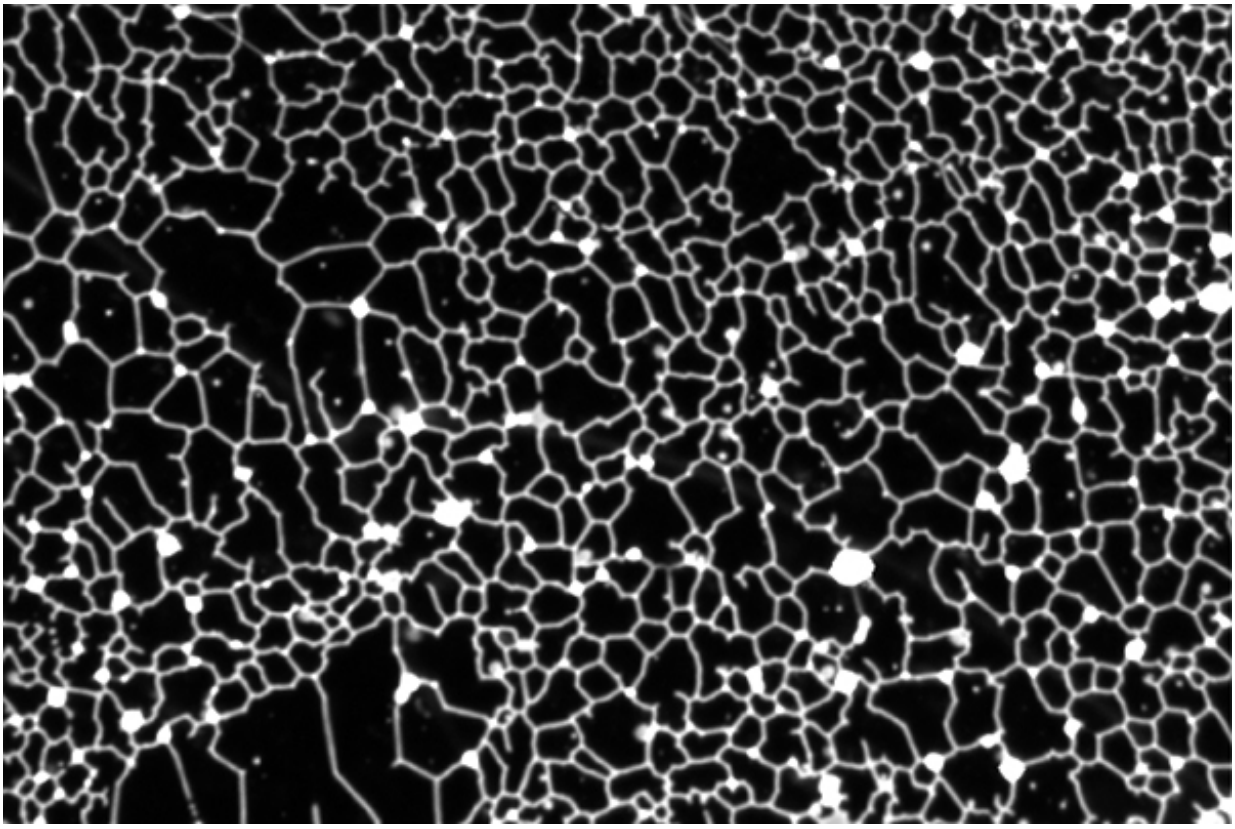
Researchers determine how part of the endoplasmic reticulum gets its shape

February 23 2017, by Stephanie Dutchen



Researchers have determined that just three ingredients are needed to form the endoplasmic reticulum's complex network of tubules. Credit: Robert Powers and Songyu Wang

From the double membrane enclosing the cell nucleus to the deep infolds

of the mitochondria, each organelle in our cells has a distinctive silhouette that makes it ideally suited to do its job. How these shapes arise, however, is largely a mystery.

Harvard Medical School cell biologists have now cracked the code for part of the endoplasmic reticulum (ER), a protein- and fat-making organelle that consists of stacked sheets in some parts and a complex network of tubules in others.

Producing the ER's tubular network is "surprisingly simple," requiring just three ingredients, principal investigator Tom Rapoport, professor of cell biology at HMS, and colleagues report Feb. 22 in *Nature*.

In addition to answering a long-standing question about basic biology, the findings help explain how certain genetic mutations in ER proteins lead to hereditary spastic paraplegias, progressive muscle disorders of the lower limbs.

"Explaining a disease doesn't mean we can cure it, but it's at least gratifying that we can trace back a complex group of diseases to a molecular defect of individual proteins," said Rapoport.

Forming the ER's tubular network involves two proteins that are engaged in a constant tug of war to maintain the right amount of tubule curvature, the researchers found.

"Now we can really understand the function of each protein," said Songyu Wang, a postdoctoral fellow in the Rapoport lab and co-first author of the paper.

Twenty years in the making

Rapoport has wanted to know for two decades how organelles get their

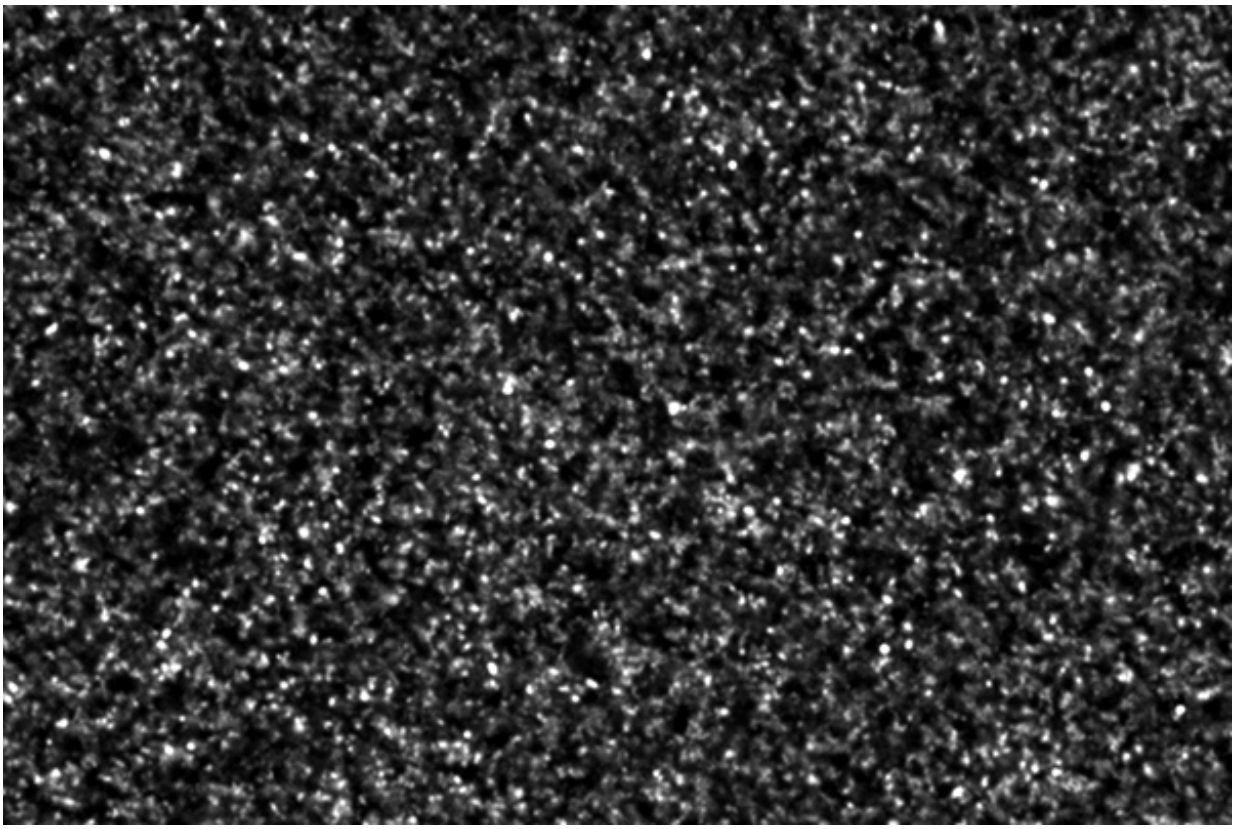
shapes. Over the years, his lab has assembled the pieces of the puzzle for the ER.

Tubules are highly curved, with O-shaped cross-sections. The Rapoport team's first discovery was identifying the proteins that stabilize this curvature. The researchers found that either of two protein families can do the job: reticulons and REEPs (receptor expression-enhancing proteins).

But one tubule does not a network make; the tubules must fuse with one another. Next, Rapoport's team figured out that enzymes called GTPases help the tubule membranes stick together.

Researchers in a different laboratory discovered another protein, lunapark, that they believe stabilizes the junctions between connected tubules, although Rapoport conducted follow-up experiments that didn't convince him.

Was this handful of molecules enough to generate a tubule network, or were there more players? So far, the research had been conducted in whole cells or in frog egg extracts, where it was hard to rule out contributions from any of the thousands of other proteins floating around. So Rapoport's team decided to build an ER network from scratch in a simpler system.



Individual vesicles (frame one) transformed into the ER's distinctive tubular network (frame two) when the researchers added a curvature-stabilizing protein. Credit: Robert Powers and Songyu Wang

"You can't start doing this kind of experiment until you're almost certain you have all the components," Rapoport said. "If it doesn't work, you don't know whether you're missing a component or whether you screwed up the reconstitution."

Networking, ER-style

Rapoport and team began by purifying the proteins they'd identified and generating artificial membranes called liposomes that contained only

those proteins.

"Purifying membrane proteins is not a trivial thing. Doing reconstitution experiments with liposomes is even harder," said Rapoport. "I don't think there are many other labs in the world that would have been able to do it. I'm proud of it. You know, this is the kind of work we're known for."

The team first inserted a yeast GTPase, Sey1, into the liposomes. Instead of a network, the ER formed small spheres, or vesicles.

Then the team added GTP, an energy-producing molecule that GTPase acts on. The vesicles got a little bigger, but still no network.

Finally, the team added a REEP family protein, Yop1p, to the mix. A network took shape.

"It took some long days and nights on the microscope, so it was nice to see something as beautiful as this network come out of it," said Robert Powers, a graduate student in the Rapoport lab and co-first author of the study. "It is a striking image when you see it for the first time."

Blocking the GTPase after network formation made the network dissolve, suggesting that the fusion and curvature-stabilizing proteins actively counter each other.

"The REEPs or reticulons are kind of overdoing it. They generate more curvature than they actually need to," Rapoport explained. "When you don't have the fusion activity of Sey1, then the REEPs or reticulons win and break up the network."

Rapoport's team went on to show that vertebrates may need even fewer ingredients than yeast: a fruit fly GTPase, atlastin, took care of both

fusion and curvature stabilization, eliminating the need for a REEP or a reticulon.

"I've always been fascinated by problems where you start with a complex biological system and then narrow it down," said Rapoport. "That, for me, is the greatest satisfaction, when you can explain a system that initially looks intractable."

The role of lunapark remains to be discovered, as does how the ER's stacked sheets take form.

More information: Robert E. Powers et al. Reconstitution of the tubular endoplasmic reticulum network with purified components, *Nature* (2017). DOI: 10.1038/nature21387

Provided by Harvard Medical School