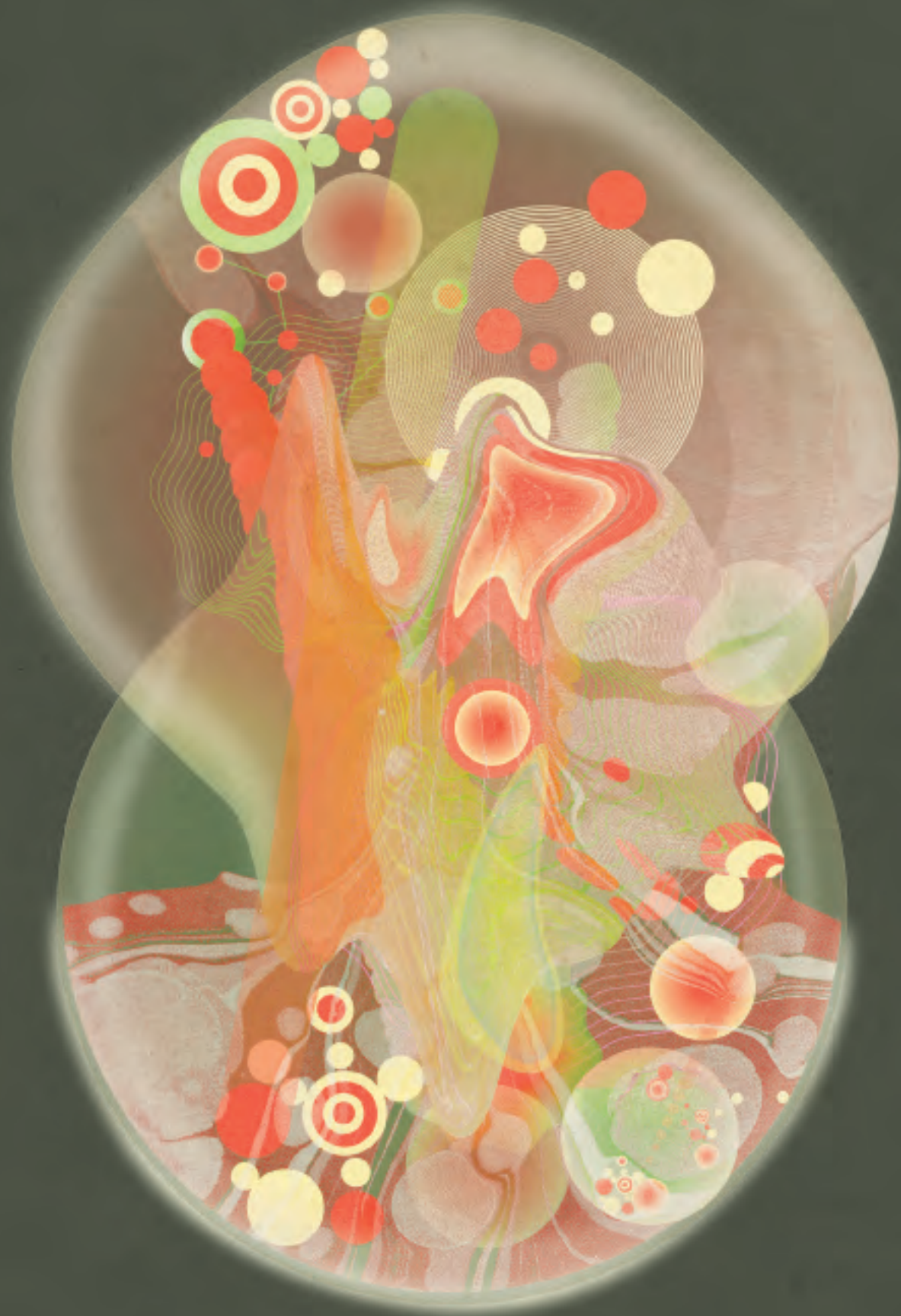


A Matter of Equilibrium

BY SARAH C.P. WILLIAMS

Researchers are
getting at the
cell's busy internal
membranes
by studying
human diseases.

ILLUSTRATION BY EMILY FORGOT



On a computer screen, two empty bubbles float toward one another.

They're made of membranes, like those in living cells. When the digitized membranes touch, molecules in each begin bobbing and shifting. Soon, the membranes merge, forming one larger sphere where once there were two. It's a slow-motion computer simulation of one of the most hard-to-visualize processes inside living cells: membrane fusion.

In a living organism, the fusion—as well as the separation, or fission—of membranes happens constantly: when a sperm fertilizes an egg, when HIV enters an immune cell, when neurons release neurotransmitter. Yet the molecular details of this vital process are hard to nail down. Membrane fusion is fast, and scientists can't freeze membranes in the act of merging. They can, however, take a step back and look at links between membrane traffic and human diseases.

From birth defects to neural degeneration, when a cell can't control its membranes, the effects are severe. Studying the diseases that result from disorganized membranes has illuminated basic biochemical processes that keep membranes at equilibrium.

Moving Parts

The outer surface of a cell is a membrane, and membranes also divide a cell's contents into discrete areas. Like a house with rooms dedicated to different purposes—eating, sleeping, cooking—a cell has compartments for different processes—building proteins, storing chemical messengers, generating energy. The walls of the cell's rooms are membranes.

When material in a cell needs to move from one spot to another, it moves

in a vesicle, a membrane-enclosed sac. Randy Schekman, an HHMI investigator at the University of California, Berkeley, focuses on vesicles that transport newly made proteins out of the endoplasmic reticulum (ER), which is itself a maze of membranes. The ER is involved in synthesizing, folding, and transporting proteins produced by the ribosome. But not all proteins are destined to leave the organelle. The vesicle-creation machinery is selective, picking off the ER assembly line only proteins that display certain chemical signals—like the zip codes a post office needs to ship packages.

“There has to be a way of ensuring that only a restricted set of proteins are transported from one place to the next,” he says. “Otherwise everything would blend.” [Cells have a system for sending wayward proteins from the Golgi back to the ER. See Web Extra “Return to Sender” at www.hhmi.org/bulletin/feb2010.]

The vesicles that transport proteins from the ER to their next destination—the Golgi apparatus, a stack of membranes where proteins are further processed—are called COPII vesicles. A specific set of proteins coat their surface. Schekman has characterized these COPII coat proteins, showing that one of them, called sec24, decides whether proteins leave the ER in vesicles. Human cells have four variations

of sec24 and each recognizes different proteins. If no sec24 variant recognizes a protein, that protein remains in the ER.

That can cause a problem during organism development, according to a recent collaboration between Schekman and David Ginty at the Johns Hopkins University School of Medicine.

In 2008, Schekman got a call from Ginty, an HHMI investigator who studies, among other things, the development of the neural system. A graduate student in Ginty's lab had been studying an extreme form of spina bifida, in which the neural tube fails to close during fetal development. In his search for gene mutations leading to this birth defect, the student turned up sec24b—one of the four sec24 variants.

Neural tube closure during development relies on a precise gradient of molecules to distinguish areas of the fetus. Together, Ginty and Schekman's labs revealed that without sec24, one of these molecules, vangl2, remains stuck in the ER and never establishes the gradient the cell banks on. Though earlier fetal development can progress with a, c, and d variants of sec24, neural tube closure stalls without sec24b. The results were published online in *Nature Cell Biology* on December 6, 2009.

Schekman's group is also exploring ER management of a macromolecule that is

PART 2 OF 2

In Part 1 of this two-part series, readers learned about the action-packed outer membrane of the cell (see *HHMI Bulletin*, November 2009).



Tom Rapoport, Randy Schekman, and David Chan have opened a new window into the membranes within the cell—including vesicles, endoplasmic reticulum, and energy-generating mitochondria—by studying diseases of the metabolic and neural systems.

important to human health: cholesterol. When a cell doesn't need cholesterol, it stores SREBP, a protein involved in cholesterol production, in the ER. When the cell requires cholesterol, vesicles transport SREBP out of the ER, allowing it to turn on cholesterol-producing machinery. Current cholesterol-lowering drugs block that final production machinery, but Schekman would like to see drugs that block the creation of the vesicles that carry SREBP out of the ER in the first place. His lab is collaborating with HHMI investigator David Ginsberg at the University of Michigan to identify which sec24 variant wraps SREBP in membranes and to determine how to block the interaction between SREBP and that variant.

"There is really an increasing overlap between trafficking in the cell and human metabolic diseases," says Schekman. All forms of hyperlipidemia—including high cholesterol in the blood—relate to how membranes move lipid molecules around, he says. Brain disorders may also involve cell membranes (see Web Extra "Dropping the Payload" at www.hhmi.org/bulletin).

Shape Shifting

At times, the cell requires a more monumental shift in membranes than protein

transport by vesicles. Sometimes an entire organelle needs to move or change shape or size. Unlike most houses, designed with immovable walls, cells can rearrange their insides as needed.

Tom Rapoport, an HHMI investigator at Harvard Medical School, wants to know how cells achieve this fluidity. His team has probed the biochemistry of various membrane channels; now they're dabbling in questions of membrane architecture—and are seeing connections to disease.

The lab group specifically studies how the cell generates the elaborate network of interconnected sheets and tubules that make up the ER. The ER is sheet-like nearest the nucleus of the cell and consists of more tubules near the periphery of the cell. In different stages of the cell's life cycle, the balance of sheets and tubules changes. Scientists are only beginning to understand how it happens.

In 2006, Rapoport identified two families of proteins—reticulons and DP1s—that shape the lipids of the ER into tubules. In a test tube, lipids mixed with these proteins spontaneously arrange themselves into tubules. More recently, in an August 2009 paper in the journal *Cell*, Rapoport and his collaborators pinpointed a protein—atlastin—that causes fusion

between tubules. This mechanism could be responsible for generating a tubular network and may well be what the cell uses to shift its balance of tubules and sheets, says Rapoport.

Scientists have linked a mutation in atlastin with a neurological disease: hereditary spastic paraplegia, characterized by progressive weakness and stiffness of the legs. The disease is caused by shortening of axons, the long slender fingers that project outward from neurons to relay messages from the body's extremities to the brain.

Rapoport's study linking atlastin to tubule fusion—and more specifically, ER fusion—offers an explanation for spastic paraplegia. Without ER fusion, it's likely that the ER network in long neuron cells can't extend far. "If the ER network is not extending all the way," says Rapoport, "that's causing problems at the ends of the axons."

Interestingly, an analogous problem in plants has been linked to defects in ER morphology. Plants with a mutation in a protein that functions like atlastins have short wavy root hairs in places where the roots should be long. In both cases, the ER's inability to mold its membranes has tremendous consequences.

(continued on page 48)

CONTINUED FROM PAGE 29
(THE CHINA CONNECTION)

he cautions that “the new traditions of high-quality science have yet to become established at most of the Chinese research institutes. My concern is that most institutions need to move more quickly in the direction of merit-based resource allocation and promotion. Rigorous review of the research performance of individual scien-

tists rarely happens, and the outcome of the reviews, if they were carried out, rarely has any consequence.”

While Xu believes that far more progress needs to be made, he is generally optimistic about China’s “tremendous potential” in science. “Scientific interaction is one of the best ways to deepen the understanding between China and America,” he says, looking up at his teleconference screen with its

live connection to his colleague in Shanghai. They are discussing how to expand the Fudan institute’s research and offer its unique mouse mutants to scientists worldwide who are trying to understand and find cures for diseases.

“This is a site where East meets West,” he says. “We are engaged in a common goal: to develop knowledge as a way to improve the well-being of humankind.” ■

CONTINUED FROM PAGE 33
(A MATTER OF EQUILIBRIUM)

For other diseases, though, the culprit is the equilibrium between the fission and fusion of a different organelle: the mitochondrion.

Mitochondria, energy-generating organelles, snake throughout the insides of cells in an interconnected network. “People often think of mitochondria as static organelles that work alone,” says HHMI investigator David Chan, “but they constantly fuse and divide. No one really understood why these events happen, or how, until the last 10 years.”

Chan, at the California Institute of Technology, studies how the cell achieves a balance in its mitochondria network. If fusion overwhelms fission, the mitochondria become excessively long and connected, eventually “collapsing into a messy jumble,” says Chan. And if fission overwhelms fusion, the organelles are dramatically fragmented and less efficient at producing energy. It’s a delicate balance.


When geneticists at Duke University linked a mitochondrial fusion gene to a neurological disease, Chan wondered how a defect in mitochondrial fusion might lead to peripheral neuropathy, which causes numbness and weakness in the hands and feet. Chan engineered mice that lacked the implicated fusion gene, *mitofusin2*. He found defects in the mitochondria of the Purkinje cells, a class of neurons in the brain known for the dramatic fan of fibers

that branch off them. With crippled mitochondrial fusion machinery, the arbor of fibers was reduced to short stumps.

Looking closer at the mitochondrial membranes, Chan saw fragmented organelles, not the interconnected network that ought to be there. Furthermore, mitochondria usually contain their own DNA—mtDNA. “But in this fragmented mutant, only a fraction of them have mtDNA,” Chan says. The observation of missing DNA explains why fragmented mitochondria can’t produce energy—they lack the DNA that encodes proteins controlling energy generation. Neurons may be particularly sensitive to these defects, because the cells are among the most energy demanding. His lab is pursuing the link between mitochondrial fission and fusion and mtDNA,

since mtDNA defects are associated with additional pathological conditions.

On a computer screen, when membrane fission and fusion are slowed down, they appear to be straightforward processes. Press play and the membranes move. The merging of membranes looks fluid and natural, like it requires no molecular machinery at all. After all, two soap bubbles can join together without the help of proteins. But inside the cell, as these researchers have shown, taking away a piece of the membrane’s control mechanisms leads to a messy jumble of membranes, or a stand-still in vesicle creation—and both problems have unmistakable links to disease. ■

 WEB EXTRA: To learn what happens when vesicles arrive at their targets and how structural biology is illuminating the biology of vesicles, visit www.hhmi.org/bulletin/feb2010.



Subscribe!

Knowledge Discovery Research Education

These four key components of HHMI’s work also guide and define the mission of the Institute’s quarterly magazine, the *HHMI Bulletin*.

Subscribing is fast, easy and free. Visit www.hhmi.org/bulletin and follow the instructions there to subscribe online.

While you’re online, read the Web edition of the *Bulletin*.

HHMI BULLETIN



This paper is certified by SmartWood for FSC standards, which promote environmentally appropriate, socially beneficial, and economically viable management of the world’s forests.