THERE IS NOTHING STATIC OR RANDOM ABOUT THE INSIDE OF A CELL. MINISCULE MOLECULAR CITIES ARE CRISSECORDED WITH ROADS DOTTED WITH VEHICLES ON THE GO; FLUID COMPONENTS MERGE AND SEPARATE. EVERY MOVEMENT IS DELIBERATE—CAREFULLY PACKED SPHERES REPETITIVELY TRAVEL THE SAME WELL-WORN ROUTES, AND CARGO IS DELIVERED TO THE RIGHT PLACE TIME AND AGAIN. THE SIGNALS THAT GUIDE THE SPHERES, OR VESICLES, ARE HARD TO SEE, BUT THEY ARE THERE.

FOR THE THREE WINNERS OF THE 2013 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE, NO ROAD SIGNS GUIDED THEIR CAREER PATHS, AND NO ONE HAD FORGED THE TRAIL AHEAD OF THEM. IN FACT, EACH SCIENTIST, OVER THE SPAN OF A FEW DECADES, BEGAN STUDYING HOW CELLS DIRECT MEMBRANE-BOUND VESICLES SPECIFICALLY BECAUSE HE SAW IT AS AN UNTOUCHED AREA OF BIOLOGY.

“I WAS LOOKING FOR SOMETHING THAT WAS NOVEL AND UNEXPLORED AND IMPORTANT ALL AT ONCE,” SAYS HHMI INVESTIGATOR THOMAS SÜDHOF OF STANFORD UNIVERSITY. SÜDHOF, HHMI INVESTIGATOR RANDY SCHEKMAN OF THE UNIVERSITY OF CALIFORNIA, BERKELEY, AND JAMES ROTHMAN OF YALE UNIVERSITY DEVELOPED NEW METHODS TO STUDY CELLS AT THE SAME TIME THEY UNCOVERED THE MOLECULES THAT CONTROL THE FORMATION, TRAFFICKING, AND FINAL DISASSEMBLY OF VESICLES.

ON THEIR WAY


“PEOPLE WERE REALLY NOT LOOKING AT MEMBRANES FROM A MECHANISTIC AND MOLECULAR PERSPECTIVE AT ALL,” SAYS SCHEKMAN. “SO I THOUGHT THAT, GIVEN MY TRAINING AND INTEREST, I COULD MAKE A CONTRIBUTION.”

THREE YEARS INTO THAT EXPLORATION, IN 1978, SCHEKMAN SAYS HE HAD HIS ONE TRUE “EREKAE!” MOMENT, WHICH PAVED THE WAY FOR ALL HIS SUBSEQUENT WORK. WITH A GRADUATE STUDENT, PETER NOVICK, HE WAS GROWING CULTURES OF YEAST CELLS WITH RANDOM MUTATIONS, HOPING TO FIND MUTANTS THAT FAILED TO TARGET VESICLES, POINTING TOWARD A GENETIC KEY TO VESICLE BIOLOGY. ONE AFTERNOON, NOVICK WAS EXAMINING A NEW MUTANT THAT STOOD OUT.

“I REMEMBER VIVIDLY TO THIS DAY WHEN PETER CALLED ME OVER TO THE MICROSCOPE,” SCHEKMAN SAYS. “THE CELLS WERE POCKMARKED WITH ACCUMULATED VESICLES.”

THE CELLS, IT TURNED OUT, HAD A MUTATION IN A GENE THEY CALLED SEC1. WHILE THE CELLS COULD FORM VESICLES, THE VESICLES WERE UNABLE TO FUSE WITH THEIR DESTINATION MEMBRANE TO RELEASE THEIR CONTENTS. SCHEKMAN HAD A STARTING POINT FOR

THE WINNERS OF THIS YEAR’S NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE DISCOVERED HOW PACKAGES OF MOLECULES ARE FERRIED AROUND AND BETWEEN CELLS.

BY SARAH C.P. WILLIAMS
his molecular studies on membrane trafficking.

In the following years, the lab uncovered mutations representing 23 different SEC genes. Each blocked membrane traffic at a different stop light: Some were deficient in forming vesicles at the Golgi complex, an organelle that packages proteins to be secreted out of the cell. Some blocked vesicles at the endoplasmic reticulum. And some mutants, like SEC1, failed to allow vesicles to fuse with the cell’s outer membrane. As a result, fully packed vesicles accumulated in the cell.

When asked what’s been key to his success at unearthing so many components of the system—more than 50 genes now—Schekman doesn’t hesitate. “Being adventurous and willing to gamble at something new and different,” he says. “Scientists don’t do that enough.”

By this time—the 1980s—Rothman, then at Stanford, had also turned his attention to the choreographed movements of vesicles. His goal: to reconstitute in a beaker all of the components necessary for vesicle fusion. Whereas Schekman’s approach was genetic, Rothman’s was protein based.

“Very distinguished cell biologists cautioned me to not bother because it would never work,” Rothman wrote in a 2002 *Nature Medicine* paper on his career.

But Rothman came up with a cell-free system that worked quite well, allowing him to follow the movement of radioactively labeled molecules from one membrane-bound compartment to the next within a Golgi complex. By studying his new system with electron microscopy, he elucidated even more details. He saw that the transport vesicles were enveloped in a coat of molecules, now known as a COP coat. And by disabling GTP, one of the cell’s energy currencies, his lab group could keep this COP coat from disassembling at the vesicle’s destination. Like Schekman, he’d discovered a molecular cause for a vesicle traffic jam.

By the 1990s, Rothman had discovered another key component of vesicle trafficking. Three SNARE proteins formed a complex that tethered transport vesicles to their destination and spurred the unzipping of a vesicle from the target membrane. One, synaptobrevin, was attached to the outside of vesicles themselves, while the other two, SNAP-25 and syntaxin, were found on the target cell’s outer membranes.

As it turned out, many of the proteins that Rothman had discovered were encoded by genes that Schekman had pinpointed. The two researchers, each drawn to the little-studied world of membrane trafficking, had helped map the molecular signals that control the traffic inside the cell.

**Brain Traffic**

Vesicles aren’t just for schlepping molecules around the interiors of cells, however. They also carry messages from cell to cell. In the brain, for example, vesicles full of neurotransmitters control the passage of signals from neuron to neuron. The proper functioning of these vesicles is responsible for a person’s every thought, action, and memory. Südhof has spent 25 years studying how these vesicles fuse with such precision and speed as to keep up with the brain.

When a neuron receives an electrical impulse, the charged pulse travels down the length of the long cell to
"The longer I’ve worked on membrane trafficking, the more apparent it has become that it’s incredibly important."
—THOMAS SÜDHOF

the presynaptic terminal, the end that reaches toward a neighboring neuron. At this terminal, calcium floods into the cell through channels that open in response to the impulse. Then, vesicles that are positioned, ready and waiting, at the presynaptic terminal fuse with the outer membrane and release neurotransmitters into the synapse, the space between the neuron and its neighbor. The neurotransmitters fill the synapse and bind to receptors on the membrane of the next neuron. In response to this binding, the cycle of neuron excitation begins in the second cell, where vesicles containing neurotransmitters will eventually fuse with the outer membrane, passing a signal to a third cell. The process continues as a message moves through the brain.

When Südhof launched his career, originally at the University of Texas Southwestern, he, like Rothman, had an ambitious goal: to purify and clone every protein in the presynaptic terminal, the area of the neuron where the vesicles gather. He spent more than a decade on this project, isolating one protein at a time. The growing list of components soon started to overlap the work of Rothman and Schekman—the same trio of SNARE proteins that Rothman had helped identify were present in the presynaptic terminal. They attached vesicles full of neurotransmitters to the terminal’s membranes and helped initiate the fusion of the vesicle with the membrane.

“I think the biggest surprise has been the overall simplicity of the machinery that controls this,” Südhof says. “I find it amazing that there’s only a handful of proteins that do the job.”

Südhof had another question, though: How does the flood of calcium into a neuron trigger the fusion of vesicles with the neuron’s membrane? Since the 1990s, Südhof’s lab group has studied this interaction between calcium and the SNARE proteins that control vesicle fusion.

“The work of mine that’s most often mentioned are those initial studies,” says Südhof. “But some of what I consider my lab’s most important work has occurred in the past 15 years. It’s required technologies that have only recently been developed.” A combination of genetics, electrophysiology, and mouse behavioral assays has been key to pushing his work forward, he says. His team discovered that, before a calcium influx, vesicles are docked at the membranes of neurons, attached by a partially assembled complex of SNARE proteins. When calcium enters the cell, proteins called synaptotagmin and complexin respond to the calcium molecules and cause conformational changes to the SNARE proteins. Within microseconds, these changes trigger vesicle fusion with the membrane.

Right Turn
More recently, Schekman and Südhof have steered their careers in a new direction—using their findings on membrane trafficking to understand human diseases. Schekman’s work has already been enormously helpful in the biotech world; today, one-third of human insulin is produced by yeast. Schekman was further inspired at an HHMI scientific meeting where he heard a presentation on Alzheimer’s disease. “It became clear to me that there were issues of membrane traffic that may be malfunctioning in genetic forms of Alzheimer’s,” Schekman says.

He’s shifted nearly his entire lab from studying membrane trafficking in yeast cells to studying the process in human cells—a huge undertaking, he says, that he wouldn’t have been able to spearhead if not for his HHMI support.

And Südhof realized that a number of the genes involved in the formation of the presynaptic terminal and the presynaptic vesicles have been implicated in autism and schizophrenia. So his lab is taking a closer look at these disease-causing mutations. “I think that neuropsychiatric disorders are an enormous challenge right now, and this is a new angle we can look at,” Südhof says. “The longer I’ve worked [on membrane trafficking], the more apparent it has become that it’s incredibly important.”

Südhof and Schekman, both HHMI investigators for more than 20 years, say their career paths are testaments to the incredible power of basic research. If there’s a broader message that they want to convey as Nobel Laureates, that is it. “I have a concern that we are losing appreciation for studies that simply give us facts—the need to describe something in detail and dissect its components in order to understand how it works,” says Südhof.

Schekman, who was editor-in-chief of the Proceedings of the National Academy of Sciences from 2006 through 2011 and is now the editor-in-chief of the open-access journal eLife, agrees: “Basic science is the foundation on which all technologies and medical applications are based,” he says. “And I worry that young scientists are moving away from pursuing this kind of essential basic science.”

He hopes that, if anything, his success offers a road sign that early career scientists will follow: Studying a basic biological system and charting your own path can lead to great things.