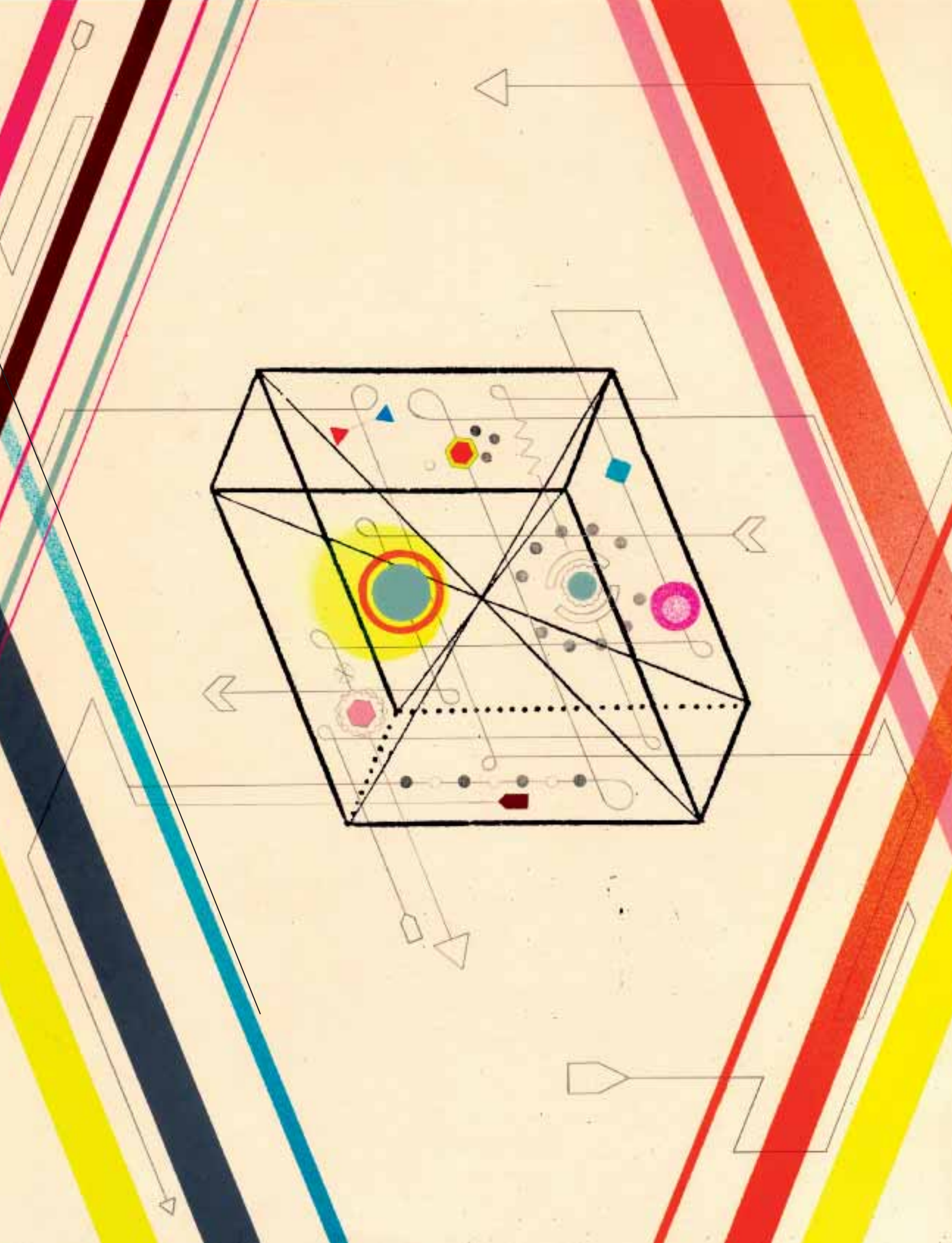


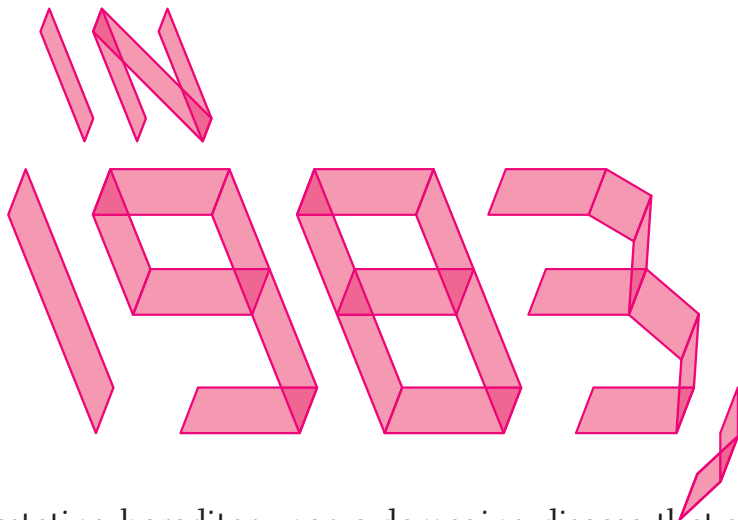
STUDYING THE FORM AND  
FUNCTION OF THIS CELLULAR  
SAC OF ENZYMES IS LEADING  
TO INSIGHTS ON DISEASE—  
AND A CLEARER PICTURE OF  
HOW CELLS ACHIEVE THEIR  
COMPARTMENTAL DESIGN.

# PARSING PEROXISOMES

BY DAN FERBER

ILLUSTRATION BY MARK ALLEN MILLER





a bright, good-natured five-year-old named Lorenzo Odone began to slur his words, throw temper tantrums, fall down, and lose his hearing. His doctor diagnosed him with cerebral adrenoleukodystrophy, or ALD,

a devastating hereditary nerve-damaging disease that strikes young boys. The prognosis was grim: Within a year, cerebral ALD robs boys of their eyesight, hearing, and ability to move and speak; most die by age 14.

Desperate to save their son, Lorenzo's parents, Augusto and Michaela Odone, worked tirelessly to develop a dietary supplement that suppressed the body's overproduction of a dangerous fat that destroyed the myelin sheath insulating the neurons. In the 1992 movie *Lorenzo's Oil*, Hollywood portrayed that supplement—the boy's namesake oil—as a miracle cure. The reality is more complicated.

Hugo Moser of Johns Hopkins University, the researcher portrayed pseudonymously in the movie as the impersonal Professor Nikolais, and Ann Moser, his wife and research partner, had studied ALD since the 1970s. After the Odones developed Lorenzo's oil, the Mosers conducted a decade-long prospective clinical trial to test its efficacy. By 2004, they'd found that the oil can prevent the disease from progressing, but it can't reverse the damage already done. Perhaps, the Mosers reasoned, an early screening test could spot ALD kids soon enough to enable the oil to hold the disease's devastation at bay. They began a determined pursuit of such a test.

The protein that's mutated in ALD patients is housed in an unappreciated organelle in the cell called the peroxisome. Defined only in the late 1960s, peroxisomes are small and spherical and distributed throughout the cell's watery interior, or cytoplasm. Most peroxisomes, which are found in fungi, plants, and animals, including humans, enclose enzymes that carry out several reactions, including breaking down certain lipids and making others, such as the plasmalogens that maintain the myelin sheath.

As the Odones' story unfolded in the late 1980s, much about the peroxisome was a mystery. But now, thanks to a small contingent of researchers, the organelle has begun to give up its secrets.

Recent advances in peroxisome biology have generated hope for treating diseases, such as ALD, that involve a single crippled peroxisome enzyme. Some researchers are even developing treatments for more devastating diseases in which the peroxisome never forms correctly, such as Zellweger syndrome, which is uniformly fatal during infancy, and infantile Refsum disease with its progressive nerve damage.

Peroxisome research has also served up insights about the fundamental workings of eukaryotic cells, which make up the tissues of all higher organisms. Eukaryotic cells are organized into organelles and other compartments specialized to carry out different functions. By studying peroxisomes, scientists are getting the first exciting glimpses of how proteins are shipped across biological membranes, how organelles are formed and maintained, and how they are retooled during development. But that's just the beginning, says HHMI investigator Randy Schekman of the University of California, Berkeley, who adds that peroxisome biology is a field "about to break wide open."

#### **PEROXISOME PARTS**

Peroxisome research received a much-needed boost in 1989 when Wolf Kunau of Ruhr University in Germany developed a way to isolate peroxisome-deficient mutants in yeast—and reveal the genes relevant to peroxisome activity. Yeast need peroxisomes to digest lipids but not to digest sugar. Kunau's team took advantage of this characteristic, screening for mutant strains that could grow on sugar but not on a lipid component called oleic acid. Kunau

and other researchers then looked for genes that, when added back to the mutant yeast strains, restored their ability to grow on oleic acid. Today 23 of those genes are known to play a key role in forming a working peroxisome.

Not long after Kunau's discovery, geneticist David Valle, a former HHMI investigator at Johns Hopkins University, was editing a book chapter on peroxisomes. He spoke extensively with Hugo Moser and became fascinated with the organelle. It was the early 1990s, and Hugo and Ann Moser had already amassed and characterized a collection of cultured skin cells from hundreds of patients with ALD and other hereditary peroxisomal diseases. In cells from less severely afflicted patients, intact peroxisomes could be observed by treating cells with antibodies to the peroxisome surface; subsequent biochemical tests on the cells revealed a single defective peroxisomal enzyme. In cells from the sickest patients, however, the researchers saw peroxisome ghosts—empty sacs with none of the enzymes the organelle usually contained.

Valle's team, including then-postdoc Jutta Gärtner, began working with the Mosers and a Hopkins colleague, Stephen Gould, to identify the genes that go awry in the sickest group of patients.

For about eight years, starting in the mid-1990s, they took genes that Kunau and others had found were needed to form peroxisomes in yeast and searched for equivalent human genes in a sequence database. When they got a match, they isolated the candidate human gene and added it to one of the Moser's cultured skin cell lines to see if it restored working peroxisomes. Nine did. Today, mutations in 13 different genes are known to cause human peroxisome biogenesis diseases. Those genes provided a parts list for the human peroxisome, albeit an incomplete one. Fundamental questions remained.

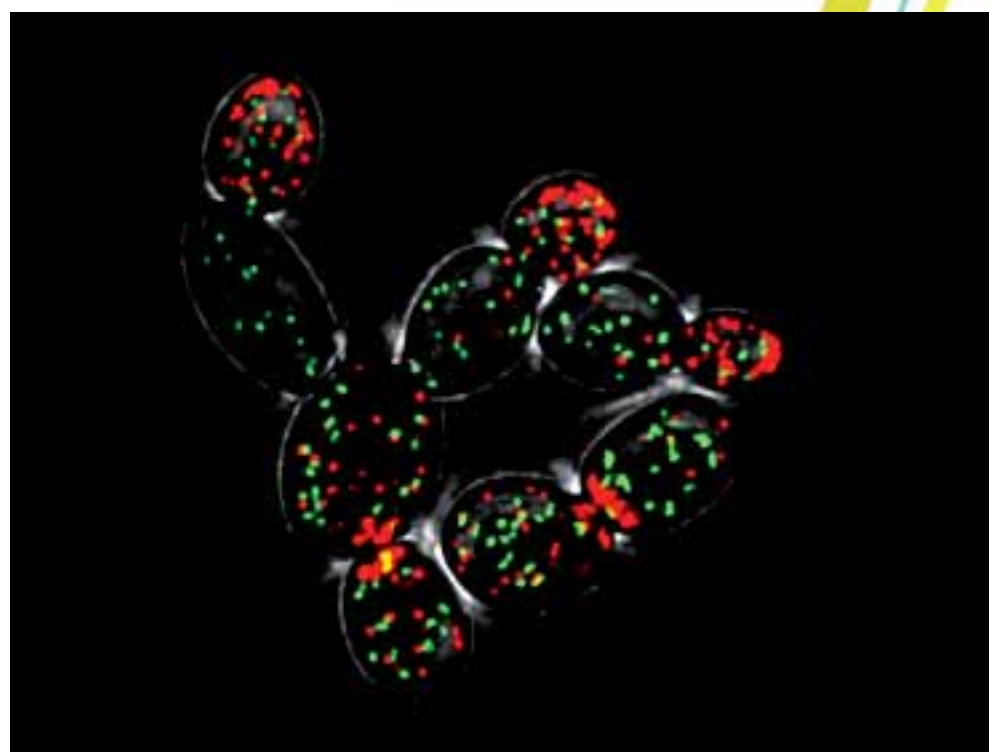
### WHENCE THE PEROXISOME

One such question was: how is the organelle formed? Dogma had it that new peroxisomes formed when existing ones divided—the same way that mitochondria reproduce. But cell biologist Richard Rachubinski, an HHMI international research scholar at Canada's University of Alberta, believed otherwise. His team kept finding evidence that peroxisomes were produced by the endoplasmic reticulum (ER), a network of

flattened sacs resembling a stack of pancakes that helps the cell make membranes and package proteins for shipment out of the cell. In yeast studies, they found one type of immature peroxisome that seemed to be budding from the ER. They noted that two peroxisomal proteins were covered with sugars that are attached to protein only when they move through the ER.

Others in the field were skeptical. When his team submitted papers to journals, reviewers demanded control after control, so many that one paper ballooned to more than 60 figures. "We got roasted, constantly," Rachubinski recalls.

The debate raged for more than a decade. Then, in 2005, Henk Tabak, a cell biologist at the University of Utrecht, the Netherlands, created a hybrid protein—half peroxisomal membrane protein called Pex3, and half green fluorescent protein from jellyfish. In cells with the hybrid, green spots clustered first on the membrane of the ER. The green clusters would then bud off as a vesicle and mature into a normal (albeit green) peroxisome. The green peroxisomes formed only in the presence of Pex19, a protein known to be



The surprising news that peroxisomes are generated in the endoplasmic reticulum came from studies in yeast. Here, in *Yarrow lipolytica*, peroxisomes are green, and the actin proteins that help usher them around the cell are red.

required for peroxisome assembly, the researchers reported in *Cell*. In an accompanying commentary article, Schekman recapped the popular view that peroxisomes were autonomous and then added that, “the authors of cell biology textbooks may wish to reconsider this view when they write their next edition.”

Later that year, Rachubinski’s group reinforced the case for peroxisomes emanating from the ER. First they created a hybrid protein similar to Tabak’s with a part of Pex3 attached to green fluorescent protein. The hybrid protein first accumulated at the ER and then formed green peroxisomes, but only when intact Pex3 protein was present in the cell.

Then they conducted a test to see if the ER-derived peroxisomes behaved normally. They created a hybrid of thiolase, an enzyme that normally sits inside peroxisomes, and a red fluorescent protein. Without intact Pex3 around, the red thiolase scattered throughout the cell’s cytoplasm. In the presence of intact Pex3, however, the newly formed green peroxisomes soon turned yellow, indicating that thiolase had moved in—and that the ER-derived peroxisomes behaved as they ordinarily did. “That was very, very cool,” he says.

More recently, Rachubinski’s team reported at the American Society for Cell Biology meeting in December 2007 that shutting off production of two ER proteins in yeast blocks peroxisome formation. “We believe there is special ER machinery” that gives rise to the peroxisome, Rachubinski says.

Now he and others, including Schekman, are sussing out that machinery. Schekman suspected that, by studying how peroxi-

some form at the ER, he could uncover a mechanism by which cells move material around in vesicles. Over the years, his team had helped characterize the cell’s best-known secretion pathway, known as the SEC pathway, by which the ER packages proteins into vesicles for shipment to the Golgi apparatus, which processes them and directs them to the cell membrane to move out of the cell. Evidence suggested that peroxisomes are formed through a different mechanism, Schekman says. For example, mutations that block normal ER protein secretion don’t affect ER-derived peroxisome production.

To deduce how the ER produces peroxisomes, Schekman’s team has created a yeast cell extract that can, in a test tube, produce vesicles that may be peroxisome precursors. They are fishing around in the extract to find the partner proteins that work with Pex19 to get the ER to produce vesicles that form peroxisomes. “Until now people thought there was one avenue of egress from the ER to the Golgi apparatus for secretion. Now it’s clear that the ER feeds the growth of other organelles in the cells—certainly the peroxisome and I bet others.”

The work should shed light on how individual membrane proteins are directed to different destinations in the cell, Schekman says. “That underlies how the eukaryotic cell achieves its compartmental design.” Compartmental design allows several complex biochemical reactions to take place at the same time, a phenomenon that makes higher forms of life possible.

#### TRAFFIC FLOW

Throughout the 1990s, Kunau and others identified cellular workhorse proteins that the peroxisome needs to function and probed how they worked together. Peroxisomes look like water balloons, with a lipid membrane and a watery interior; some proteins are embedded in the membrane, while others float inside. Every mutation that caused human peroxisomal disease blocked one protein or another from getting into the peroxisome, and two-thirds of them blocked proteins from reaching the

#### CHANGING WITH THE TIMES

Plants are the only well-documented example of an organism that remodels its peroxisomes, and HHMI professor Bonnie Bartel of Rice University has begun probing how they do it. In oilseed plants, unlike yeast and mammals, cells revamp their peroxisomes to perform new functions as the organism matures. In seeds and seedlings, peroxisomes contain enzymes that break down stored fats and produce sugar to fuel plant growth. In mature plants, those peroxisomal enzymes are eliminated and replaced with others that improve the efficiency of photosynthesis.

Bartel’s team identified a pair of proteins that may help plant peroxisomes undergo this transformation. They found that mutations in two proteins, Pex4 and Pex22, cause peroxisomes in plant seedlings to retain an enzyme that’s usually eliminated as the plants mature. That finding suggests that the two proteins help remodel peroxisomes during plant development, the researchers reported in 2005 in *The Plant Cell*.

Last summer at an *Arabidopsis* conference in Beijing, China, they reported a second enzyme that’s retained in plants with Pex4 and Pex22 mutations. Both enzymes are also retained when cells have defective proteasomes, which digest damaged proteins. Bartel suspects that, in healthy cells, Pex4 and Pex22 help mark obsolete peroxisomal proteins for removal and ship them out of peroxisomes to be destroyed by proteasomes. That, in turn, helps peroxisomes remodel as plants mature from seedlings. “As we get deeper into this, plants continue to surprise us,” Bartel says. —D.F.

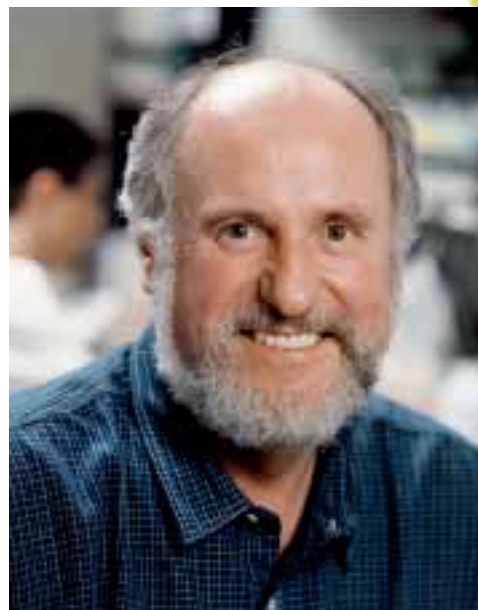
organelle's interior, Valle says. Over the past decade, Suresh Subramani of the University of California, San Diego, and his colleagues have uncovered a novel molecular machine that imports those proteins. That, in turn, has reshaped biologists' thinking about how cells direct enzymes and other types of proteins to the correct cellular organelle.

Proteins do not enter peroxisomes the same way they enter other organelles. Before being imported into mitochondria, for example, or the light-harvesting chloroplasts in plants, or the ER, a protein must unfold from its three-dimensional conformation into a long string of amino acids, which is then threaded through the organelle's membrane into its interior. But in peroxisomes, proteins move through the membrane in their folded, three-dimensional state, often escorted by partner proteins. In a series of studies that began in the mid-1990s, Subramani and his colleagues figured out how Pex5, a peroxisomal receptor in the cytoplasm, grabs a protein, escorts it through an entry gate in a large protein complex in the peroxisome's membrane, drops it off inside, and then returns to the cytoplasm through a separate exit to begin the process anew. However, receptors can get stuck in the exit gate, shutting down the entire import process, Subramani explains.

Recently, Subramani's team uncovered the RADAR pathway, an enzymatic pathway that marks receptors that are blocking the exit door and then uses proteasomes, one of the cell's "garbage disposals," to mark the receptors, destroy them, and restart the import process. "It would be fascinating to know how the cell senses and activates the garbage disposal when it's needed," Subramani says. These and other studies could shed light on how cells regulate how many of each type of organelle they keep around. Answers to that question could yield clues to how muscle cells maintain more mitochondria than other cells to supply them with extra energy, or how regulation of organelle number goes awry to cause disease.

### **FIXING FAULTY PEROXISOMES**

As other biologists uncover the complexities of peroxisomes, Valle and several former students are trying to repair those that malfunction. Sabine Weller, a pediatrician and postdoc in Jutta Gärtner's lab at the University of Göttingen, Germany, has created a mouse model of Zellweger syndrome to test therapies. Weller replaced a normal peroxisomal gene called *PEX1* with a mutant version known to cause one in three cases of Zellweger syndrome. A single amino acid substitution in the mutant allele, called *Pex1-G843D*,



The nitty-gritty of peroxisome biology is becoming clearer, thanks to a resurgence of interest in the organelle by researchers such as Richard Rachubinski (left), working with yeast, and Bonnie Bartel, working with plants (see sidebar).

causes the protein to misfold, reducing its activity to less than 20 percent of its normal capacity. This so-called knock-in mouse could be used to test drugs that might stabilize Pex1, Weller says.

Nancy Braverman, a Johns Hopkins medical geneticist, succeeded in stabilizing the Pex1G843D protein in cultured cells from Zellweger patients by cooling the cells slightly. Now she's screening thousands of potential drug compounds to find one that does the same thing. Such a compound could be tested in the knock-in mice, and possibly one day in people.

"I think there's a lot of work to be done, and treatment of genetic disease is always difficult, but this is an area where we might see some real success," Valle says.

One peroxisomal disease has already seen some limited success: ALD. Although Hugo Moser died in early 2007, Ann Moser has continued pushing for a universal newborn screening test to spot at-risk boys. Since Lorenzo's oil can prevent the disease from progressing, early detection is key. Moser and a Hopkins colleague developed a rapid blood test to detect elevated levels of very-long-chain fatty acids, a hallmark of ALD. In a small study, it appeared to be both accurate and sensitive. Now, with a Maryland state screening lab, she plans to expand the study, assessing the test on blood obtained from routine heel sticks of 5,000 Maryland newborns to make sure the screen doesn't falsely label healthy infants as sick. Moser hopes to one day put the test in place nationally. "We believe it will save families from a genetic odyssey," she says. ■