

SCIENCE OBSERVER

CELLULAR SECRETION: IT'S IN THE PITS

The crucial feature of biological membranes is their integrity, without which life could not exist. And yet the membrane of a secretory cell is breached routinely each time the cell secretes the hormones, neurotransmitters or enzymes that it makes. For decades scientists have been trying to get a handle on the mechanism of the type of cellular secretion called *exocytosis*, which involves the fusion between membranes of minute intracellular vesicles and the cell membrane. But they have been unable to actually watch this type of secretion.

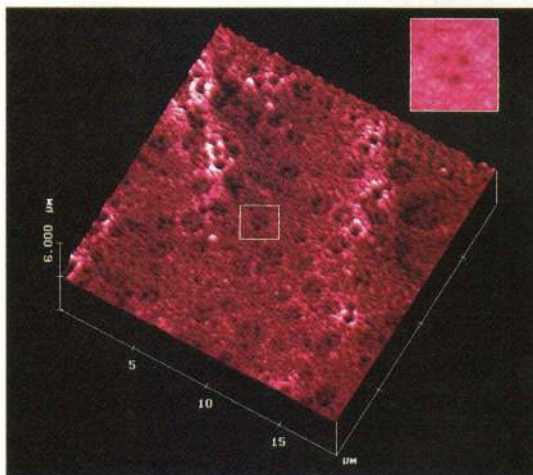
Until now. A team of scientists from Yale University and from the University of Würzburg in Germany has caught acinar cells from the pancreas in the act of secreting the digestive enzyme amylase. The group, which includes Stefan Schneider, Kumudesh Sritharan, John Geibel and Bhanu Jena from Yale, along with Hans Oberliethner from Würzburg, viewed the activity under an atomic-force microscope (AFM) and reported their results in the January 7 issue of the *Proceedings of the National Academy of Sciences* (PNAS). The work, writes Julio Fernandez of the Mayo Clinic in Rochester, Minnesota, in a commentary in the same issue, "may be the first to show a macromolecular structure undergoing conformational changes during cellular activity."

When they probed the outer surface of pancreatic acinar cells with the AFM, Jena and his colleagues found the surface to contain large crater-like pits with a number of smaller depressions inside. When the cell was stimulated to secrete amylase, these depressions grew wider and deeper for a time, and then returned to their original size after secretion stopped. Jena and his colleagues interpret the depressions to be the fusion pores, the actual site on the cell membrane where secretory vesicles dock and fuse. They believe that the changes in dimensions that they record with the AFM actually represent the opening and closing of the pores.

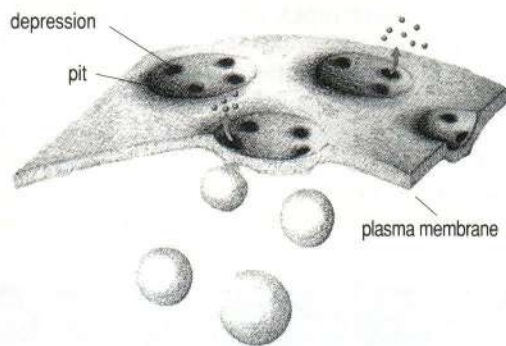
The new work aims to help scientists unravel the dynamics at the cell membrane when the secretory vesicle contacts it. "Many of us are trying to figure out membrane fusion," says John Heuser, a cell biologist at Washington

University in St. Louis. "The basic mechanism is still a total mystery."

One of the issues that biologists grapple with is whether the relationship between the membranes of the cell and of the secretory vesicle is transient or permanent. In many secretory cells—such as neurons, which secrete neurotransmitters; mast cells, which secrete histamine and serotonin; and pancreas cells, which secrete digestive enzymes such as amylase—the molecules to be secreted are



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Atomic-force microscope (top) shows the cell membrane of a secretory cell studded with large pits containing smaller depressions—the presumptive pores at which secretory vesicles dock and fuse before releasing their contents to the extracellular medium, as shown in the diagram. (Microscope image reprinted with permission from PNAS 94:316–321.)

packaged in small membrane-bound vesicles. When the cell is stimulated by an external signal, the membranes of these secretory vesicles dock on and fuse with the cell's membrane. A pore is formed at the fusion site, through which the vesicle releases its contents into the extracellular space.

In the classical view of membrane fusion, obtained from early electron-microscopic work of George Palade and others, the vesicular and cellular membranes appear to merge permanently, and the entire contents of the vesicle are emptied into the extracellular space. Because many secretory vesicles fuse with the cell membrane following a stimulus, the cell membrane temporarily incorporates a substantial amount of vesicular membrane. At some later point, this incorporated vesicular membrane is thought to be recycled back into the cell in a process called *endocytosis*, at which time the secretory vesicle is refilled, reformed and ready to respond to another stimulus.

Soon after this scenario was proposed, evidence for a second, more transient mechanism started to crop up. In 1984, Fernandez and his colleagues published the results of their electrophysiological studies on histamine release from mast cells, which showed that some of the vesicles fuse only briefly with the cell membrane, release only a fraction of their contents into the extracellular space and then retract and reseal. "Kiss and run" is what biologists have nicknamed transient fusion, which not only requires less of a commitment from the membranes but also uses up less metabolic energy than does permanent fusion. The new work, notes Jena, provides additional support for transient fusion.

"There are still a large number of people who believe that fusion is an all-or-none event," says Fernandez, but he adds that different modes may be used in different cell types, or even in the same cell under different circumstances. In the mast cells he studies, Fernandez estimates that about 20 percent of the fusions are transient. Having two modes is undoubtedly an attractive idea, says Heuser. In situations where cellular secretion has to be most rapid, such as in neuronal communication, the prediction would be that the "kiss and run" mode is favored, whereas for longer secretory events, ones that might involve memory, for example, scientists might expect fusion to be permanent. Nevertheless, says Heuser, his own work suggests that this is not so. In frog neurons that stimulate muscle cells, where

neurotransmitter release is known to be extremely rapid, he and his colleagues have observed that "vesicular fusion is essentially irreversible," he says. Jena, on the other hand, believes that exocytosis is primarily a transient event, with total fusion taking place only when the contents or components of the vesicular membrane need to become incorporated into the cell membrane.

The issue of transient versus permanent fusion aside, there remain several questions about what exactly Jena and his colleagues saw under the AFM. The use of this type of microscope is relatively new, and its potential for imaging biological structures in three dimensions is enormous, says David Clapham of Harvard. Clapham and his colleagues have used the AFM to image both the opened and closed conformations of the pore on the nuclear membrane of frog egg cells. Unlike Jena's group, however, Clapham and his coworkers looked at fixed rather than live cells. The problem with the AFM, he notes, is the difficulty in interpreting the images.

The AFM works by probing the surfaces of the objects it is imaging, be they cells or crystals. A hard probe is attached to an arm, called a cantilever, and the imaging process, notes Fernandez in his commentary, is like a phonograph needle riding over the surface of a record. Dips and bumps in the surface of the material being imaged cause the cantilever to deflect, and the deflection is registered as a force. The problem in imaging cell membranes, notes Fernandez, is that they are elastic and easily deformable. Probing a membrane with a cantilever is like probing the surface of a block of Jell-O with a chopstick. If the chopstick pushes into the Jell-O, one might get a false impression of dips in the surface.

For that reason, Fernandez suggests that Jena and his team are probably not producing direct images of the pore through the membrane. "The AFM will report only the hardest components of the cell, those associated with the cytoskeleton," the network of proteins that gives the cell its shape and structure, says Fernandez. In particular, he says, Jena's team is probably imaging the "cytoskeletal structures that support the fusion pore," an assertion that has experimental support also reported in Jena's *PNAS* paper. Whether or not Jena's group is seeing the actual pore, Fernandez says the work shows that something is changing with time, and that is "interesting, no matter what it turns out to be."—Michelle Hoffman

INVISIBLE WATERMARKS

The 5th-century B.C. Greek historian Herodotus told a tale in which one of his countrymen, Histiaeus, needed to send a secret message to a fellow soldier. To do so, Histiaeus shaved the head of one of his slaves, tattooed the message on his scalp and waited

until the slave's hair grew back. Then he sent the slave to his fellow soldier, who was told to shave the slave's head—unveiling the hidden message. That encryption technique is a form of *steganography*, or covered writing. In today's world of digital images, which can be easily used without