Cell Biologists Explore 'Tiny Caves'

Recent work suggests that the small membranous structures called caveolae are specially designed for getting molecules into the cell and are possibly also centers for signal transduction

Any vehicle small enough to drive over the cell's outer membrane would surely have a bumpy trip. The cell's surface is hardly smooth and featureless like an interstate highway. In fact, it's more a gravel-strewn back road—studded with pits and indentations. But unlike potholes, the cellular indentations are far from nuisances. Take the type called "coated pits" because their inner surfaces are covered by a dense layer of the protein clathrin. These coated potholes play an essential role in receptor-mediated endocytosis, a process in which the cell carries proteins and other large molecules into the cytoplasm.

For many years, most of the attention of biological road crews was focused on the coated pits. Now, though, it's beginning to look as if another, much less studied, membrane indentation—the caveolae ("tiny caves")—may one day rival the importance of their clathrin-coated counterparts. Within the past year or two, researchers have shown that caveolae also draw substances into the cell's interior, although in caveolae's case the transported substances are smaller molecules, such as vitamins. There's also growing evidence that the caves participate in signal transduction, the complicated process by which extracellular stimuli like growth signals are transmitted into the cell. Some of the most enthusiastic biologists speculate that defects in the function of caveolae may even be connected to cancer and other illnesses.

These results are causing a strong upswing of interest. Caveolae "have been seen in the electron microscope for over 40 years and only now are we beginning to understand some of the secrets behind their function. It's very exciting. I think there's a whole new field getting started," says cell biologist Richard Anderson of the University of Texas Southwestern Medical Center in Dallas.

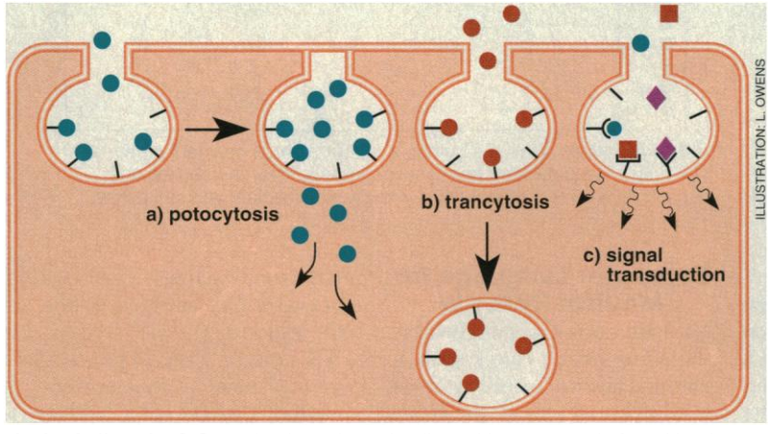
Excitement like that was certainly not on the agenda for caveolae back in the 1950s, when they were observed, as little more than dimples in the cell's outer membrane, and named by Japanese electron microscopist Eichi Yamada. Further microscopy showed that they were more than simple invaginations in the cell. They were in fact formed by small vesicles, around 50 nanometers in diameter, fused to the cell membrane. Those micrographs, however, gave researchers few clues to what caveolae actually do.

The earliest functional hypothesis was proposed by cell biologist George Palade, now at the University of California, San Diego, whose group noticed these structures around the same time as Yamada. Because Palade detected caveolae in the endothelial cells lining the walls of blood vessels, he suggested that they might be vehicles for a process called "transcytosis," in which blood-borne molecules are picked up and transported across the endothelial cell to the membrane on the other side where their contents can be released into the fluids bathing the cells of the tissues. Subsequent studies showed that radiolabeled molecules that were picked up by the caveolae did, in fact, make their way across endothelial cells as Palade proposed.

"Only now are we beginning to understand some of the secrets behind their function."
—Richard Anderson

Despite such evidence, the transcytosis hypothesis has been controversial for decades. For starters, no electron microscopist could capture a picture of closed caveolae in transit across the cell—which, in fairness to Palade's idea, could have been because they move too fast to be detected or because the tissue preparation methods used in electron microscopy made the structures fuse to the membrane. But there were other uncertainties about caveolae's activities: Their presence in cell types other than endothelium cells suggested that they might have functions beyond transcytosis, but what those functions might be was unclear.

The main problem handicapping research into caveolae was that investigators were limited to looking at micrographs; they hadn't figured out a way to isolate these curious vesicles physically and subject them to rigorous biochemical analysis. Those problems have begun to fade in the 1990s, spurred by what Deborah Brown, a biochemist at the State University of New York (SUNY) at Stony Brook calls the "rediscovery" of caveolae by Anderson's lab. Anderson and his colleagues have been studying the cytoplasmic surface of caveolae, which in electron micrographs shows up as a series of concentric ridges and furrows. More important, through antibody labeling of cells, they discovered that caveolae have a membrane marker protein, analogous to the clathrin that covers the coated pits, which they called caveolin. At the time, which was early last year, they even suggested caveolin might help isolate caveolae—a prediction that may already have been fulfilled.



Jack of all trades? Caveolae have been implicated in potocytosis for taking in small molecules, transcytosis for transport across cells, and signal transduction.

Anderson's lab, however, was interested in more than caveolin. They concentrated much of their effort on a class of molecules known as GPI-anchored proteins, so named because they are anchored to the cell membrane by a lipid called glycosylphosphatidylinositol (GPI). Anderson's group found that GPI-anchored proteins, whose functions more and more biologists are growing curious about, cluster in caveolae. One such protein is the receptor for the B vitamin folic acid, which is present in very low concentrations in the blood stream. While studying caveolae in kidney epithelial cells, the Anderson

group discovered that after the folate receptors in caveolae bind the vitamin, caveolae close off their extracellular openings and can then dump a tiny flood of folate into the cytoplasm.

From this evidence, they proposed that caveolae are responsible for a new form of cellular uptake that they label potocytosis, with "poto" coming from a Latin verb that means "to drink." Unlike endocytosis, in which coated vesicles break free from the membrane and actually move into the cytoplasm, in potocytosis caveolae remain tethered to the membrane, closing their extracellular opening and thus creating a high concentration of ions that encourages their crossing the caveolae's membrane into the cytoplasm. Potocytosis appears to be the first clear-cut role for caveolae and Anderson contends many other small molecules and ions other than folate are delivered into the cell this way. Indeed, he suggests that concentrating and supplying dilute molecules to the cell is the central function of caveolae.

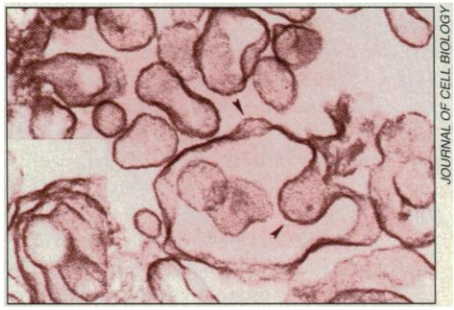
Not every cell biologist accepts that claim, however. Some are more than a little wary, contending that not even all epithelial cells make use of caveolae this way. "If potocytosis exists, it does not apply to endothelium," says Palade, who still maintains that transcytosis will ultimately prove to be one of caveolae's primary duties.

The apparent ability of caveolae to concentrate molecules that bind to receptors may be useful for more than just cellular uptake of metabolically necessary molecules; it also points to a role for caveolae as crucial sites where incoming signals from hormones, growth factors, and other extracellular regulatory molecules are brought into the cell. Michael Lisanti of the Whitehead Institute for Biomedical Research goes so far as to call caveolae "signaling organelles."

Lisanti and other investigators are now providing the experimental evidence to support this provocative notion. In recent experiments, for example, Kyoto University's Toyoshi Fujimoto and his colleagues in Japan labeled cells growing in culture with antibodies to identify proteins that concentrate in caveolae. They found enriched amounts of both a pump that removes calcium from the cell and a protein called the inositol trisphosphate receptor, whose function may be to let calcium ions into cells. Since the caveolae contain machinery used in both calcium influx and outflux, Fujimoto has proposed that their role is to regulate calcium ion concentrations in the cytoplasm, a crucial task since calcium ions control many cell activities.

Other evidence implicating caveolae in signal transduction comes from an entirely unexpected source. In order to study cell structure, researchers for decades have washed cells with detergents such as Triton-100, which dissolves almost everything except the fibers of the cytoskeleton. This method, however, does leave behind insoluble protein-lipid complexes that can be separated from the cytoskeletal materials. Researchers have usually discarded these complexes as waste, but Whitehead's Lisanti now argues that they are isolated caveolae.

Lisanti began studying these complexes because they are rich in GPI-anchored proteins, his area of expertise. Earlier this year, he and his colleagues took electron micrographs of the insoluble material and got a surprise. The micrographs revealed spherical structures, 50 to 100 nanometers in diameters, that displayed surface features remarkably like those of the caveolae. That's when Anderson's identification of a marker protein like caveolin became crucial. Lisanti's group found that the concentration of that protein is 160 times higher in the complexes than in whole cells, clinching the case that they are actually caveolae, says Lisanti.



Caving in. The arrows point to membrane invaginations known as caveolae.

Through studying the molecules found in these detergent-insoluble complexes, Lisanti has become convinced caveolae play a major role in cell signaling. The complexes proved to be a rich source of proteins involved in various signaling pathways. In addition to those that had already been identified as being associated with the caveolae, Lisanti has found more than a dozen new ones. "We think caveolae are the region a lot of cell surface receptors will go to signal. I would bet more than half of the signaling molecules we know about will be there," he says.

Lisanti is already trying to trace at least one signal transduction pathway through the caveolae. He suggests caveolae may be the site where growth control signals are relayed to the Src family of tyrosine kinase enzymes. These enzymes add phosphate groups to other proteins, and in doing so, propagate growth control and other messages throughout the cell. Lisanti's research has shown that caveolin is a transmembrane protein, and he speculates that it may serve as the intermediary between GPI-anchored proteins and Src-like kinases. As Lisanti sees it, a ligand would bind to a GPI-anchored protein that in turn would transmit a signal to caveolin and from there to Src-like kinases.

That idea is not far-fetched. In the past 2 years, a number of investigators have obtained evidence indicating that many of these kinases do associate with GPI-anchored proteins. Moreover, the kinase produced by the *src* oncogene has been shown to phosphorylate caveolin. That might disrupt the normal processing of growth signals by caveolae, says Lisanti, and explain how oncogenic Src causes the unrestrained cell growth of cancer.

If Lisanti is right about the caveolae being centers for many signal transduction pathways, disruption of their function might well lead to other deleterious consequences. Some investigators caution, however, that caveolae's role in signal transduction is still in the realm of speculation. Much of it rests on the belief that the complexes Lisanti and others have isolated are in fact caveolae. But not everyone is convinced of that yet. "We're not automatically calling these detergent-insoluble complexes caveolae," says SUNY's Brown. She warns that washing cells and tissues with detergents like Triton is a harsh technique that may eliminate some key molecules that would normally be part of caveolae in the cell.

As investigators make further comparisons between proteins found in the complexes and those localized to caveolae by antibodies, it should become clear whether they have actually gotten their hands on isolated caveolae. Even assuming they have, cell biologists envision years of work before they understand the mechanics and role of these cellular vesicles. Most believe that caveolae will eventually be exposed as jacks-of-all trades, serving multiple functions, perhaps within the same cell. "A lot is riding on these little pits," comments Doug Lublin of University of Washington. "This area is new enough that we're trying to define the structure at the same time we're trying to define its functions." It may be many years before biological spelunkers reveal all the riches buried in these little caves.

–John Travis

Additional Readings

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