What's in the Vault?

An ignored cell component may often account for why chemotherapy fails

By JOHN TRAVIS

Can you imagine exploring the anatomy of the human body and missing the heart, the organ that sends life-giving blood coursing through the body? Of course not. Or not noticing the brain, the custodian of memories and creator of thoughts? Don't be ridiculous.

Yet cell biologists may soon have to acknowledge an equally unimaginable oversight in their field. For decades, their powerful microscopes have failed to spot a basic cell component of animals and perhaps any organism with a nucleus. Known as vaults, the barrel-shaped particles are three times the size of ribosomes, the easily seen protein-making factories of cells.

Vaults were unearthed 10 years ago only by accident, even though they exist by the thousands in the cells of rats, humans, chickens, sea urchins, and even slime molds. Almost as surprising, a decade after they were spotted, vaults remain largely a mystery, their role uncertain and their existence disregarded by most cell biologists.

Investigators may not be able to ignore these obscure objects much longer: Cancer researchers have identified a tantalizing link between vaults and the frequent failure of chemotherapy to destroy tumors.

Nancy Kedersha laughs when remembering how she stumbled upon vaults in the mid-1980s. Then at the University of California, Los Angeles (UCLA), Kedersha and her colleague Leonard H. Rome were studying coated vesicles, protein-covered fatty spheres that convey molecules around the interior of cells. Kedersha was struggling to purify these microscopic moving vans, carefully separating the coated vesicles from other contents of the cell. "I wasn't trying to discover anything. I was just trying to clean up my coated vesicle preparation," recalls the cell biologist.

Kedersha turned to negative staining, a microscopy method as simple as it is messy. When using an electron microscope, biologists normally dust cells with chemical stains intended to highlight the contents. In negative staining, they flood their samples with stain.

If her preparation contained only stained vesicles and the stain-filled fluid around them, Kedersha would view a sea of black when she looked at it through a microscope. But if it were contaminated with objects that shrug off the stain, that sea would be dotted with white islands. Rome likens the strategy to finding an invisible person by looking for an unexplained shadow in the beam of a spotlight.

To Kedersha's surprise, unstained ovoid objects appeared among her coated vesicles. Since some of the stain settled into furrows on top of the unexpected shapes, the negative staining revealed fine details of the exterior of these mysterious interlopers, including arches that reminded Rome and Kedersha of the ceilings in medieval cathedrals. The two investigators thus christened the curious items vaults.

The researchers quickly discovered why they, and other scientists, had never noticed vaults before. The stains used in imaging cells generally latch onto fatty molecules in the membrane of a cellular component or mark the nucleic acids that make up DNA and RNA. But vaults consist almost entirely of proteins, which traditional stains leave untouched. "In transmission electron micrographs, they're practically invisible," says Rome.

Since they reported the existence of vaults in 1986, Kedersha, now at the Cambridge, Mass., biotech firm ImmunoGen, and Rome have developed a detailed account of these objects. The main constituent of vaults is a protein called the major vault protein (MVP). An individual vault is apparently built of 96 copies of this protein. RNA is another integral, though hidden, part of vaults. Each appears to contain 16 short RNA strands tucked inside of the barrel-like container created by the major vault proteins.

Measuring some 55 nanometers by 30 nanometers, vaults sometimes look in microscopic images like pairs of unfolded flowers, each half of the vault made of eight petals attached to a central ring by a small hook. Those images suggest that vaults open and close as a natural part of their function in the cell, says Rome.

The researchers have precious few clues to the role of vaults in the cell. The best lead comes from their unique shape. "I'm a firm believer in form following function. Nature is trying to tell us something by this incredible structure. And the one thing we might surmise from the structure [of vaults] is that they might contain something," says Rome.

That shape also hints that vaults may pick up their unknown cargo at the nuclear membrane, the barrier that separates the cell's cytoplasm from its nucleus. The nucleus is a fluid-filled sac containing DNA and the machinery required to translate the instructions encoded by that DNA into molecules called messenger RNA. These mRNA strands, as well as other molecules, must somehow get out of the nucleus. The portals they use are membrane structures called nuclear pore complexes. Remarkably, says Rome, vaults match almost perfectly the size and shape of pores formed by these complexes.

Furthermore, some researchers have observed what they call plugs filling nuclear pore complexes. While Rome acknowledges that many investigators discount the existence of plugs, labeling them experimental artifacts, he believes that plugs may be the same as vaults. Some images of vaults, he notes, show them lolling around the cytoplasm in the vicinity of nuclear pore complexes.

"It's a perfect match to me. My opinion is that vaults either dock at the pore complex or dock at the pore complex and are the plugs. I think they're moving things from the nucleus into the cytoplasm," says Rome. Suggesting one type of cargo, Rome says that brief strands of vault RNA may serve as attachment sites for mRNA, which the vaults would then ferry around the cell.

Vaults might have languished in obscurity for many more years, studied only by Rome, Kedersha, and a few other adventurous souls, if it were not for a discovery made last year by a group of researchers led by Rik J. Scheper of Free University Hospital in Amsterdam. Until recently, Scheper and his colleagues were oblivious to the existence of vaults. The group had focused its research efforts on cancer, particularly the troublesome phenomenon of tumor cells that can escape destruction by chemotherapeutic drugs.

Such drug resistance frequently causes chemotherapy to fail, says Scheper.

Many forms of cancer are either naturally unyielding to drugs or develop resistance in the course of therapy, probably because tumor cells mutate into resistant forms that survive and proliferate.

In the last few years, investigators have begun to unravel the molecular mechanisms that guard cancer cells from drugs. They have discovered that some cancer cells resistant to several commonly used drugs dramatically increase the production of proteins that pump various drugs out of a cell's interior. In particular, two recently identified proteins, P-glycoprotein (Pgp) and multidrug-resistance-associated protein (MRP), serve this protective function.

Not all cancer cells depend upon Pgp or MRP. Since 1993, Scheper's group has investigated a protein that many drug-resistant lung cancer cells produce in unusual abundance. Early indications are that this protein, known as lung-resistance-related protein, or LRP, may be the most effective predictor of whether a particular cancer will respond to chemotherapy, says Scheper.

His group recently joined forces with researchers at the National Cancer Institute to examine a large variety of cancer cells stored there. Production of LRP was found in 78 percent of the cancer cells, notes Robert H. Shoemaker of NCI's Developmental Therapeutics Program in Frederick, Md. The presence of LRP, more so than that of either Pgp or MRP, provided the most accurate indication of whether the cells were susceptible to chemotherapeutic drugs, the researchers report in the Jan. 17 INTERNATIONAL JOURNAL OF CANCER.

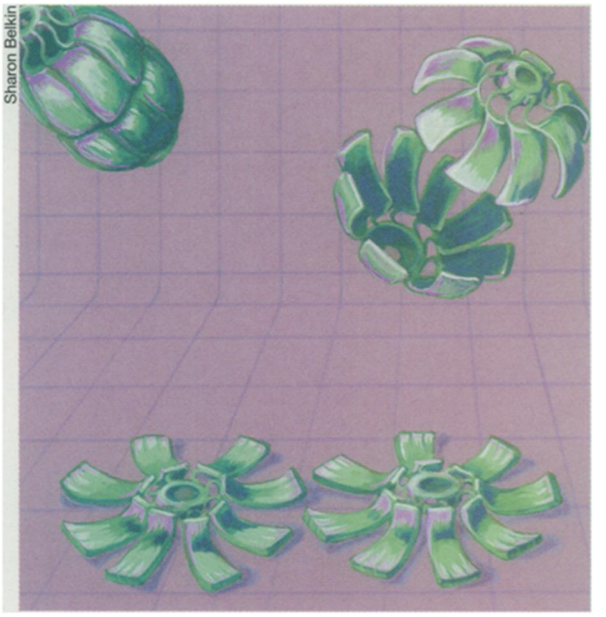
Furthermore, Scheper and his colleagues have examined the tumor cells of people with ovarian cancer or acute myeloid leukemia. In both types of cancers, the investigators found that people whose tumors made LRP had not responded well to chemotherapy or survived as long as people whose tumors had no LRP. "It looks like [LRP production] is a predictor of a poor response to chemotherapy, but it's still too early for regular clinical screening," comments Scheper.

Scheper's research took an extraordinary turn last year, when his group finally found the gene that codes for LRP. That gene turned out to be the gene for the human version of the major vault protein. "It shocked us," says Rome, recalling his reaction when he first learned of Scheper's discovery, later reported in the June 1995 NATURE MEDICINE.

The connection between vaults and drug-resistant cancer cells gained more support earlier this year. Since the synthesis of LRP may not in itself mean that a complete vault forms, Rome's and Scheper's groups joined together to examine the number of actual vaults in drug-resistant cancer cells. At the RNA Society meeting in Madison, Wis., Valerie A. Kickhoefer, a UCLA colleague of Rome's, reported that drug-resistant cancer cells do indeed make more vaults than other cancer cells do—as much as 16 times the normal amount.

How vaults may confer drug resistance upon cancer cells remains a matter of speculation. If vaults transport molecules, especially if they ferry compounds away from the nuclear membrane, cancer cells may employ them to oust DNA-damaging drugs from the nucleus or to convey other toxic drugs away from their intended targets elsewhere in the cell.

Scheper cautions that no one has yet proved that vaults are responsible for drug resistance in cancer. That, investi-



These are an artist's renditions of vaults. Whether these unusual structures transport any cargo remains a mystery.

gators agree, would require proof that the elimination of vaults from resistant cells robs them of their protection or that the addition of vaults to susceptible cells confers resistance.

While the identification of LRP as the major vault protein suggests that cancer cells can commandeer vaults for their own ends, the discovery doesn't resolve the lingering mystery of what vaults do in normal cells. Rome still holds that vaults move about the cytoplasm, periodically docking at nuclear pore complexes to pick up strands of mRNA for transport. Yet Kedersha has evidence that vaults may also dwell inside the nucleus.

Moreover, she and Kathy Suprenant of the University of Kansas in Lawrence champion an alternative theory about the cargo of vaults. They note that vaults are far larger than any mRNA they might carry. "You don't need anything this big to move mRNA around," says Kedersha.

Instead of carting mRNA, Kedersha and Suprenant suggest, vaults may help form and haul the two subunits that make up ribosomes, the organelles that translate the information encoded by mRNA into strings of amino acids.

Suprenant was led to join the small band of scientists studying vaults by her research on microtubules. These hollow filaments crisscross the interior of a cell and provide it with support. Working with cells from sea urchins, Suprenant and her colleagues recently isolated unusual complexes that contain microtubules, ribosomes, other proteins, and mRNA. One protein in these complexes turned out to be the sea urchin's version of the major vault protein. Prompted by that discovery, the investigators took a closer look at the complexes. "Lo and behold, there are vaults," says Suprenant.

Moreover, the vaults are intimately associated with the ribosomes from the complexes. "If you purify vaults from the preparation, there are ribosomes. If you purify ribosomes, there are vaults around," says Suprenant. She and her colleagues have also found that antibodies to the sea urchin's vault protein stain the cell's nucleolus, the site inside the nucleus where the two subunits of ribosomes form.

To Suprenant and Kedersha, the circumstantial evidence linking vaults to ribosomal assembly and transport is compelling. "Ribosomal subunits are assembled inside the nucleus, then they exit the nucleus in a manner that's completely unknown. Presumably, they go through the nuclear pore complex, because how else could they get out? There must be something that takes them across the membrane. It turns out vaults have an interior that's the right size to shuttle the ribosomal subunits across the nuclear envelope," remarks Suprenant.

Rome remains unconvinced by the arguments of Suprenant and Kedersha, noting that estimates of the vaults' interior volume are only speculation based on their exterior size. Moreover, no one has found a complete vault particle inside the nucleus, counters Rome.

As this collegial debate shows, the most rudimentary questions about vaults remain unanswered a decade after their discovery. Do vaults actually transport something, and if so, what? Where do vaults go in the cell? These pressing questions should be answered more quickly now that vaults have been associated with cancer.

"We'll get a whole new group of people anxious to find out what vaults do," predicts Rome. □