Research on the Golgi apparatus still shows plenty of life on the cell structure's 100th birthday, as researchers debate anew just how it transports proteins through the cell

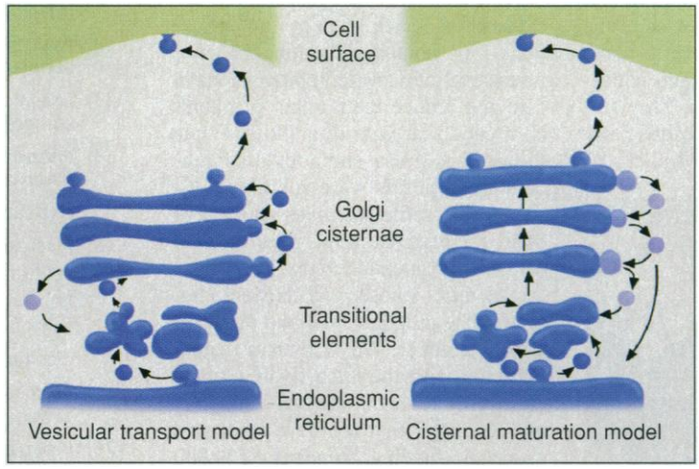
Coming to Grips With The Golgi

PAVIA, ITALY—It's been exactly 100 years since the Italian biologist Camillo Golgi, working at the university in this medieval Lombardy town, peered through his microscope at spinal neurons and saw the stack of membranous structures that now bears his name. Over the decades since then, cell biologists have learned that this membranous network plays a key role in processing and transporting newly synthesized proteins. But a conference* recently held here to celebrate the centennial of the Golgi apparatus showed that, despite the organelle's venerable age, research on it is still lively. Indeed, new work is forcing cell biologists to reevaluate some of the key concepts produced by the past 10 to 15 years of work on the Golgi.

The challenges don't concern so much what the Golgi does, but rather how it does it. Cell biologists have established that the structure, which consists of stacks of flattened membrane sacs called cisternae surrounded by smaller membranous tubules and round sacs, or vesicles, chemically modifies and sorts two types of newly synthesized proteins—those destined to be secreted from the cell, such as hormones, blood plasma proteins, and digestive enzymes, and those that function in the cell's membranous compartments. All these proteins start their life in the protein factories of the endoplasmic reticulum (ER), a membrane network that permeates the cytoplasm. Then, they move into and through the Golgi before reaching their final destinations.

Until a year or so ago, researchers thought that proteins pass across the Golgi stack in vesicles that bud off from one cisterna and then fuse with the next. But accumulating evidence from several laboratories suggests that instead of acting as static warehouses, receiving and dispatching cargo in the shuttling vesicles, the cisternae themselves may move forward while the vesicles actually move backward to recycle components of the ER and Golgi compartments to their sites of origin.

This new view of a dynamic Golgi, known as the cisternal maturation model, "means the difference between a rigidly organized and complex [Golgi] system and a much more fluid, self-correcting, and evolving one," says Hugh Pelham of the Medical Research Council Laboratory for Molecular Biology in Cambridge, U.K. This model is already forcing researchers to rethink their notions of how new Golgi stacks are produced as the cell grows or when it needs to secrete more proteins, and also of how the Golgi forms in the two daughters when a cell divides.



Two views. In the vesicular transport model (left), vesicles carry proteins through the Golgi and also transport materials back to the ER. But in the cisternal maturation model (right), proteins are transported within the Golgi cisternae and the vesicles are involved only in backward transport.

But the work may have wider implications as well. "I think these principles affect the way we think about cell self-organization," Pelham adds. He's suggesting that rather than being a machine with rigid and unchanging parts, the cell instead consists of more organic components that grow, shrink, and flow as needed.

Still, the final word about how proteins are transported through the Golgi may not be in. Cell biologist Marilyn Farquhar of the University of California (UC), San Diego, cautions that "the evidence for [cisternal] maturation is not written in stone."

Indeed, if anything is constant in the Golgi field, it is change. The cisternal maturation model itself is a revival of an idea first proposed in the 1960s, but then discarded when both electron microscopic and biochemical evidence pointed to vesicles as the carriers of protein cargoes through the Golgi. "We have returned to the ideas of the '50s and '60s but now have the ability to address the questions with new insight and techniques," says Kathryn Howell, a co-organizer of the centenary meeting and a cell biologist at the University of Colorado School of Medicine, Denver.

Traffic patterns

The first controversy in the field arose with Golgi's description of the structure, which had its skeptics—among them his contemporary and fellow Nobel Laureate, Spanish neurobiologist Santiago Ramon y Cajal. The main problem was that Golgi could see what he called an "internal reticular apparatus" only in cells stained with a heavy metal, and this staining "was difficult and capricious," writes Eric Berger of the University of Zurich, Switzerland, editor of a book that marks the centenary of Golgi's discovery. Golgi wasn't proved right until a half-century later, when cell biologists first began using the electron microscope (EM) to examine intracellular structures and saw the intricate stacks of cisternae, surrounded by small vesicles, in many different cell types.

The Golgi's role in protein transport emerged in the 1960s and 1970s when George Palade, James Jamieson, and their collaborators, then at Rockefeller University in New York City, began probing its functions. By combining EM with autoradiography, which can detect the locations of radiolabeled molecules, these researchers showed

* "The Golgi Complex: State of the Art 100 Years After Camillo Golgi's Discovery," 19 to 23 September.

that secreted proteins start their life in the ER and then enter the Golgi from "transitional vesicles" that bud from the ER near the innermost cisterna. The work also showed that the proteins exit the Golgi in vesicles that leave on the side nearest the cell membrane. The resolution of the technique was not sufficient to distinguish how the proteins move through the Golgi, however.

Meanwhile, work by several researchers had been showing that in addition to transporting the proteins, the Golgi also modifies them. Among other things, it tacks on the complex sugar chains that are attached to most secreted and transmembrane proteins. Antibodies that detect the sugar-adding enzymes further showed that some of them are present in only one or two distinct cisternae and that their location in the stack corresponds with the position of the sugars they put on in the complex chain.

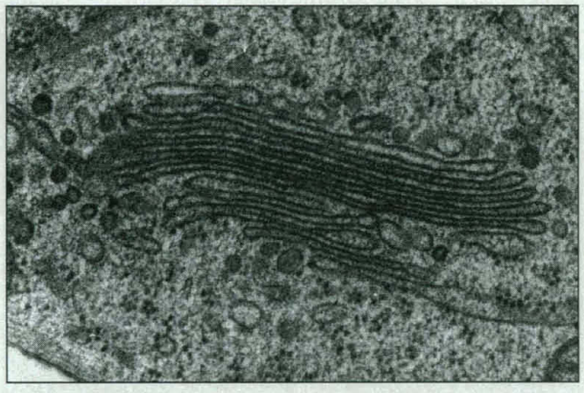
Until then, cell biologists thought that proteins might be transported within cisternae moving through the cytoplasm from the interior of the cell toward the membrane—that is, through cisternal maturation. Experiments done in the early 1970s on certain unicellular algae, which secrete large scales that form a protective coating on the algal cells, supported that idea. The scales appeared to pass from one side of the Golgi to the other enclosed in a single cisterna. But the work indicating that each Golgi cisterna has unique components suggested a different picture for most secreted proteins. The idea emerged that, rather than maturing from one to another, each cisterna receives, modifies, and dispatches its protein cargo.

Then in 1981, Palade and Farquhar suggested how the proteins might be transported from one cisterna to the next—within the small vesicles that could be seen between and around the cisternae of the Golgi stack. This vesicular transport model was also consistent with the observations that proteins enter and leave the Golgi in vesicles, and the cisternal maturation model fell into disfavor. As for the algal scales, because they are much too large to fit into the vesicles, most researchers thought that their transport within cisternae might be just a peculiarity of an obscure organism.

No one could find a way to test the vesicular transport hypothesis until 1983, however, when James Rothman of New York's Memorial Sloan-Kettering Cancer Center and his colleagues devised an ingenious cell-free assay to measure transport of a cargo protein between Golgi cisternae. This cargo protein was a viral membrane protein produced when the virus takes over the cell. The researchers isolated Golgi stacks from infected cells; they then mixed these "donor" Golgi membranes carrying the viral protein with "acceptor" Golgi membranes prepared from uninfected cells, and with cytosol—the soluble components that make up the liquid phase of the cell—and an energy source.

The movement of the cargo protein from the donor membranes to the acceptors could be monitored because the acceptor membranes, but not the donors, contained a sugar-adding enzyme from the mid-Golgi cisternae. It added radioactively labeled sugars to the cargo protein, tagging it, but only when the two were in contact within the same Golgi cisterna. Rothman's results with the assay persuaded him that the Golgi normally shuttles proteins forward from one cisterna to the next, assembly-line style.

Not only did this assay mark the first time this kind of intracellular transport had been reconstructed in the test tube, says cell biologist Graham Warren of the Imperial Cancer Research Fund (ICRF) lab in London, but "it opened the way for a biochemical analysis of the proteins that are involved in this [transport] process." By staging the assay in purified fractions of cytosol instead of in the complete mixture, Rothman's team was able to identify several proteins that are essential for intra-Golgi transport. At about the same time, other researchers—including Randy Schekman of UC Berkeley, Peter Novick of Yale University, and Scott Emr of UC San Diego—were also pinning down the proteins needed for protein movements within the cell, in their case by using mutant yeasts defective in various steps of forward protein transport.



Close-up. The Golgi apparatus consists of a stack of flattened membranous sacs. Discoverer Camillo Golgi is at left.

Even as this picture was developing, however, there were already signs that the vesicular transport model wasn't the full story. The findings on the algal scales were still worrisome, and the large amount of membrane that the vesicles would have to be transporting to the cell surface and other destinations also presented a problem. There had to be a way to redistribute it back through the pathway or otherwise remove it from the outer cell membrane—otherwise, the cell would expand indefinitely. But no one had a clear idea of how the cell does that.

More recent evidence suggests that this might be the job of the vesicles that are supposed to be transporting proteins forward. Among the proteins identified as important in vesicular transport is one called COPI (for coat protein I), which helps form the vesicles that bud from the cisternae by forming a coat on the flat membrane that probably makes it pinch outward. In 1994, Pierre Cosson and François Letourneur, working at the Basel Institute of Immunology in Switzerland, found that COPI binds to a peptide in the tails of certain ER proteins—a so-called retrieval signal—that is necessary for returning those proteins to the ER when they escape. In effect, COPI seemed to be specialized for leading the vesicles it coats on a backward journey to the ER, not a forward journey to the cell membrane.

The idea that COPI carries proteins to the ER gained further support when the Swiss team created mutant yeast strains that were unable to retain proteins correctly in the ER, and then traced this inability to defective COPI genes. Also, Hugh Pelham's group found that the receptor for the ER retrieval signals is concentrated in COPI-coated vesicles. "It became obvious that COPI was involved in backward transport both within and from the Golgi stack," says Pelham.

The key issue then became whether COPI-coated vesicles could be involved in traffic in both directions, or whether Rothman had been mistaken in his original interpretation of the transport assay. Backward transport of the sugar-adding enzyme in vesicles would look the same as forward transport of the cargo protein.

Rothman did not attend the Pavia meeting and declined to be interviewed for this article, but in the 25 July 1997 issue of *Cell*, his team, with that of Lelio Orci at the University of Geneva Medical Center in Switzerland, reported evidence indicating that vesicle traffic in the Golgi might flow in both directions. They used antibodies to search cells for a secreted protein, proinsulin, which is transported forward through the Golgi, and another protein, the retrieval signal receptor, which is transported backward. They found that the

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two proteins are present in two different populations of COPI-coated vesicles that bud from all Golgi cisternae. That implies, the team writes, "that bidirectional transport occurs from every level of the Golgi stack."

But other researchers, some of them former students and postdocs of Rothman's, suspect that the vesicle traffic goes in just one direction: backward. For example, Joachim Ostermann, now at Vanderbilt University in Nashville, Tennessee, took a fresh look at data he produced years ago during his postdoctoral work, in which he used the Golgi transport assay. He's now noticed, he reported in Pavia, that the COPI-coated vesicles generated in the assay contained the Golgi sugar-transferring enzyme. This implies that the vesicles carry the enzyme backward to meet the cargo, rather than the other way around. After following up these findings with more experiments, Ostermann concludes, "Virtually all of the data produced from the intra-Golgi transport assay can be explained by backward transport of Golgi enzymes."

Other evidence in favor of backward transport comes from the recently completed yeast genome sequence. Researchers believe that a transport vesicle can dock to the membrane of a cisterna only if the membrane displays a protein called a SNARE. "Because the entire yeast genome has been sequenced, all the SNAREs in yeast have now been catalogued," explains Pelham, "and the only ones that are in the Golgi and essential for secretion seem to be involved in backward rather than forward vesicular traffic within the Golgi complex."

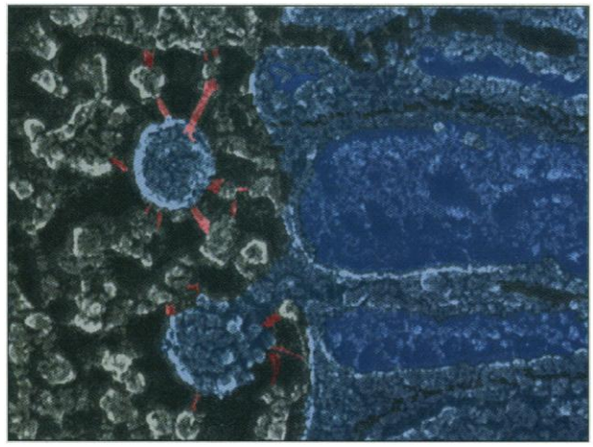
Indeed, even Schekman, who is, like Rothman, a pioneer of intracellular membrane transport research, agrees that these newer data have cast doubt on the bidirectionality of vesicular transport. "There isn't yet a smoking-pistol experiment that proves COPI vesicles are involved in forward transport through the Golgi," he says. But if proteins do not move through the Golgi in vesicles, then researchers will have to come up with another explanation. And right now, the best bet is that they are transported much as the algal scales are, by the progression of cisternae through the stack.

Some new evidence for that idea comes from Alberto Luini and his colleagues at the Consorzio Mario Negri Sud in Santa Maria Imbaro, Italy, who have been studying the transport of the large procollagen fibers needed to make connective tissue as well as bones and teeth. Like the algal scales, these fibers are much too large to enter transport vesicles, and at the meeting, Luini reported EM studies indicating they are transported by cisternal maturation across the Golgi. Also supporting the idea are EM studies from John Bergeron's team at McGill University in Quebec and others showing, in contrast to Rothman and Orci's findings about proinsulin, that secreted proteins are found only in Golgi cisternae and not in vesicles.

Because of such discrepancies, Luini, whose own work favors cisternal maturation, cautions that it is too early to rule out some role for forward vesicular transport through the Golgi. Both processes may contribute to protein transport, he says, noting that one way to find out is to compare how fast a small secreted protein and a large supramolecular structure such as a procollagen fiber move through the Golgi in the same cell. If the rates are different, he says, it may be because the transport mechanisms for the two types of protein are different.

The dynamic Golgi

But even if cisternal maturation accounts for only part of protein transport, there would have to be a way of constantly regenerating new Golgi cisternae, and that would require a revision of current views of how the Golgi forms. Until now, researchers thought that the structures are basically static once formed, and that the existing Golgi gives rise to the Golgi of the two daughter cells when a cell divides. For example, by tagging Golgi components with a fluorescent molecule, the ICRF's Warren found that during cell division it fragments into much smaller elements that disperse through the cytoplasm. These fragments are then passed down to the two daughters and reassembled, he postulates.



Coming or going? Vesicles have been implicated in both forward and backward transport through the Golgi.

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But this disappearing act looks different to cell biologist Jennifer Lippincott-Schwartz of the National Institute of Child Health and Human Development in Bethesda, Maryland, and her colleagues. When they tagged a Golgi protein with green fluorescent protein (GFP) and then treated the cells with a drug called brefeldin A, which disrupts the Golgi, they found that the fluorescence rapidly disperses throughout the ER. This indicates that the drug makes the Golgi collapse into the ER.

What's more, Lippincott-Schwartz reported at the meeting, she sees a similar, though very transient, "blink out" of the Golgi-linked GFP fluorescence in cells going through the midpoint of cell division, even without brefeldin A treatment. Like Warren, she believes that the Golgi structure fragments at the start of division. But, she proposes, all the fragments suddenly collapse into the ER, and then late in mitosis new Golgi structures begin to assemble in the daughter cells as protein-carrying vesicles bud off the ER and fuse together, eventually forming the cisternae. Such a mechanism could not only produce new Golgi structures, but also provide a way to replace them as they migrate through the stack.

Warren doesn't see this pattern, however. "We've published experiments using similar techniques, and we find that Golgi fragments persist throughout mitosis," he says.

But new data from Ben Glick at the University of Chicago add credence to Lippincott-Schwartz's story. He has been studying two yeasts, *Pichia pastoris* and *Saccharomyces cerevisiae*, both of which multiply by budding new cells off old ones, and finds that the Golgi in the yeasts do not break down during cell division. Instead, a strand of the ER enters the budding daughter cell, where it appears to give rise to a new Golgi. "If we are thinking about this correctly, then Golgi inheritance in budding yeasts is a direct consequence of ER inheritance," concludes Glick.

All in all, researchers are building a much more dynamic picture of the Golgi. Rather than being a static collection of well-defined compartments, it appears to be in constant flux, each cisterna emerging from the ER with its load of proteins and then carrying these proteins across the stack while at the same time putting the finishing touches on the proteins. That dynamism was reflected by the mood of the scientists in Pavia, who were celebrating new ideas and relishing the challenges ahead. **–CAROL FEATHERSTONE**

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ADDITIONAL READING

E. G. Berger and J. Roth, Eds., *The Golgi Apparatus* (Birkhäuser Verlag, Basel, Switzerland, 1997).

"The Golgi Complex" (Special Issue), *Biochimica et Biophysica Acta* **1404**, 1 (1998).

"Golgi Centenary Issue," *Trends in Cell Biology* **8**, 1 (1998).