

Let's make Golgi

Does the Golgi self-organize or does it form around an instructive template? Evidence on both sides is piling up, but a definitive conclusion is proving elusive.

In the battle to define the Golgi, discussions easily spiral into what can appear like nitpicking. In a contentious poster session, an entire worldview rests on whether you think a particular mutant is arrested with vesicles that are close to but distinct from the ER or almost budded from but still attached to the ER.

Sometimes obscured by these details are the larger issues. This debate “gets to the fundamental issue of how you think of the Golgi,” says Ben Glick of the University of Chicago (Chicago, IL). “The dogma has been that you need a template to build an organelle. But in the secretory system it’s possible in principle that you could get *de novo* organization of structure. That’s the issue that stirs people emotionally and intellectually.”

Then there are the collateral issues. There is an ongoing controversy about the nature of forward transport through the Golgi—it may occur via forward movement of small vesicles, or by gradual maturation of one cisterna to form the next. The cisternal maturation model “argues for a Golgi that can be made and consumed,” says Graham Warren (Yale University, New Haven, CT)—a situation that is more difficult to reconcile with Warren’s template-determined universe.

Even more confusing is the situation in mitosis. Accounts vary wildly on how much of the Golgi disappears into the ER during mitosis. The answer would determine to what extent the cell has to rebuild the Golgi after mitosis, and what method it might use to do so.

Several laboratories have made major contributions to address these

issues. But none define them so clearly as those of Warren and Jennifer Lippincott-Schwartz (National Institutes of Health, Bethesda, MD). At almost every turn, on almost every issue, it seems that Warren and Lippincott-Schwartz reach opposite conclusions, sometimes based on similar or identical data.

And yet, at least in public, there is a remarkable lack of rancor. “These are not easy experiments for us to do,” says Warren. “It’s all cutting-edge research and we are pushing the technology to the limit. Part of that is that you push your own interpretation.” For her part, Lippincott-Schwartz approaches a lengthy poster-session debate with Warren with something approaching glee. This is not triumphal glee, however. Rather, Lippincott-Schwartz seems to relish the opportunity to exchange ideas, and on this point Warren agrees. “Complacency is the worst thing to have in a field,” he says. The debate “has made all of us think a lot harder.”

In the Golgi conflict, interpretation is all.

A drug and two reversals

Brefeldin A (BFA) is a remarkable compound. This fungal metabolite is not only specific, it also has a dramatic effect on a cell biological problem that is otherwise difficult to tackle. By inhibiting an exchange factor for the small GTPase Arf, BFA makes Arf fall off Golgi membranes, thus triggering the absorption of Golgi contents back into the ER. The ability of the Golgi to recover from this treatment was the first evidence that it may be possible to rebuild the Golgi from dispersed (or more accurately, ER-localized) components.

But then Warren countered with his discovery and characterization of several Golgi matrix proteins. These proteins, such as Grasp65 and GM130, were left behind after either Triton

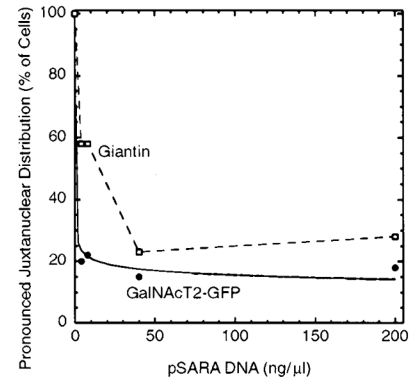


Figure 1. It takes a higher concentration of dominant Sar1 (in the GTP form) to force a matrix protein out of the Golgi region.

extraction of the Golgi, or BFA treatment. This led Warren to dub them “matrix” proteins, and to suggest that they form a scaffold for reforming the Golgi. He saw a similar pattern when he blocked exit from the ER using a mutant in another GTPase, Sar1—Golgi enzymes were stuck in the ER but the matrix proteins remained in a distinct, Golgi-like distribution.

Two papers in this issue contest the matrix interpretation. Brian Storrie (Virginia Tech, Blacksburg, VA) uses a higher concentration of the Sar1 mutant used by Warren (which is stuck in the active, GTP-loaded state) and finds that now both Golgi enzymes and matrix proteins leave the Golgi (see Miles et al. on page 543 and Fig. 1). Lippincott-Schwartz uses an alternative Sar1 mutant, this one stuck in the inactive, GDP-loaded state, to show that Golgi enzymes and matrix proteins can be forced back into the ER (see Ward et al. on page 557 and Fig. 2). In FRAP experiments, Ward et al. also show that Golgi matrix proteins can exchange dynamically rather than acting as a static matrix.

Dynamic interpretations

These experiments may disprove the extreme version of a static matrix. But they are consistent with a dynamic matrix (at least part of which is always present in the Golgi region), and they

stop a long way short of proving the counter-proposal of a Golgi that can be rebuilt after dispersal. “The only thing that has been shown is that a lot of Golgi proteins are subject to recycling,” says Tommy Nilsson (European Molecular Biology Laboratory, Heidelberg, Germany). What is needed, he says, is an experiment in which proteins are forced into and then released from the ER using a temperature-sensitive Sar1 mutant. “Then you can address whether the Golgi is a self-forming organelle or if it needs a template.”

So far it has not been possible to make a temperature-sensitive Sar1. But that hasn’t stopped the participants from coming up with other, less direct tests of their models. Perhaps most direct has been Warren’s creation of cytoplasts. These cell fragments lack a nucleus and Golgi, but retain a dispersed ER. In support of Warren’s proposed template requirement, the cytoplasts cannot form a new Golgi. Lippincott-Schwartz believes that the cytoplast ER may lack one or more components (such as Arf) that are sequestered in the Golgi. Microinjection experiments to resolve this issue are extremely difficult and as yet have not yielded any different results, according to Warren.

The cytoplasts do, however, form a new Golgi if the original cells are pretreated with BFA. Warren believes that fragments of Golgi matrix can now disperse and enter the cytoplasm, thus providing centers for Golgi reformation.

But, in her paper in this issue, Lippincott-Schwartz claims that the matrix-containing clusters seen with BFA or active Sar1 are not Golgi remnants. Based on exchange data and the presence of the CopII coat protein Sec13, she says that they are ER exit sites produced from the ER. “Once you get the result with BFA [in cytoplasts], that means the Golgi can form de novo,” she says, “because the peripheral structures are not Golgi-derived.”

Template or enzyme?

As the debate narrows to a discussion of specialized ER exit sites versus specialized Golgi fragments, what really differentiates the two viewpoints?

“Whether the system can be built from scratch from the ER is the bottom line,” says Lippincott-Schwartz. “Graham thinks there is some kind of stable element that is serving as a scaffold, and that has no enzymatic function. My view is that this can be done by self-organizing principles via an enzymatic process.”

And Lippincott-Schwartz has her own idea of what that enzymatic process might be. “What maintains the system is the activity of the Sar/CopII and Arf/CopI systems,” she says. “Sar sorts proteins out of the ER, partitions them into a subdomain. Arf allows the subdomain to completely differentiate from the ER into the Golgi. It’s a two-step process to building a Golgi.”

Lippincott-Schwartz says that the template is only needed under the original model of vesicle-based Golgi transport, in which there must be a defined acceptor compartment to greet the vesicle upon arrival. But homotypic fusion of ER-derived vesicles, followed by coat-mediated sorting of some proteins back to the ER, might achieve the same goal.

Of course, what is possible in theory is not always what occurs in practice. Warren suspects that eventually a Golgi may be able to form without a template, but this would not occur on a relevant time scale. “These are very complex organelles,” he says. “If you took all these components you must be looking at a few hundred or thousand proteins. That could take an enormous time to self-assemble. The difference between us is kinetic.” As an example of this kinetic argument Warren cites centrioles, which under certain circumstances can form de novo but whose formation is usually greatly accelerated by the seeding of an existing centriole.

The time scale of most relevance to the process of Golgi dynamics covers the period during and following mitosis. Lippincott-Schwartz believes that the

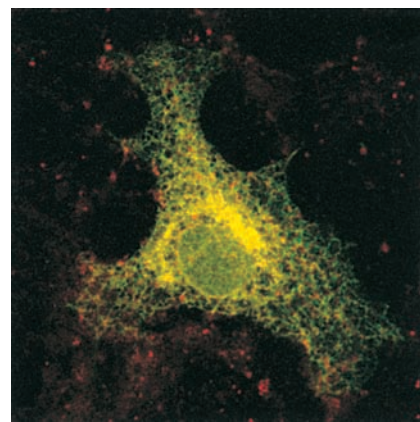


Figure 2. A Golgi enzyme and matrix protein colocalize to the ER in cells expressing an inactive Sar1 mutant.

Golgi is completely absorbed into the ER during mitosis because of an inhibition of the Sar1 and Arf systems. Warren feels many of these results were compromised by GFP fusion proteins that were retained in the ER because of misfolding, although Lippincott-Schwartz points out that her group has seen the same redistribution of endogenous proteins using antibody staining. According to Vivek Malhotra (University of California, San Diego, CA), “we don’t know where the Golgi membranes are during mitosis, and we don’t know whether Golgi can reform de novo after mitosis, especially given the time frame.”

Meanwhile, resolution of the broader template versus no-template issue may come only if the temperature-sensitive Sar1 can be made and used to generate definitive results. For now, the only agreement is on a sentiment expressed by Nilsson. “This,” he says, “is not an issue that is in any way settled.”

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