ASCB AWARD ESSAY

The Future of Golgi Research

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This essay looks backward on the past three decades of research toward understanding the mechanism of macromolecular traffic through and within the Golgi apparatus with an eye to the future. I also explain why I feel the Golgi should continue to hold the attention of molecular cell biologists.

It is an honor to be counted among those recognized by the American Society for Cell Biology with the E. B. Wilson Award and a pleasure to share this with my friends Stuart Kornfeld and Randy Schekman. Stuart taught us how complex oligosaccharides are made in an elaborate orchestration conducted successively in the endoplasmic reticulum (ER) and the Golgi and how lysosomal enzymes are selected by virtue of their special glycosylation patterns. Without this knowledge and certain key mutant cell lines that he characterized, it would not have been possible for me to reconstitute protein transport in a cell-free system. Randy pioneered the use of yeast as a model organism for discovering the machinery of the secretory pathway, from which came the first proof of the role of transport vesicles (sec1), the concept of rab GTPases (sec4), and the universality of membrane fusion (sec18/H11005) from which came the first proof of the role of yeast as a model organism for discovering the machinery of the secretory pathway, from which came the first proof of the role of transport vesicles (sec1), the concept of rab GTPases (sec4), and the universality of membrane fusion (sec18 = N-ethylmaleimide-sensitive factor [NSF]), among many other things. Randy used knowledge of Stuart’s pathway in his cell-free reconstitution, and from this came our modern understanding of ER-to-Golgi transport (coat protein II [COPII]). So we are all in their debt.

On an occasion such as this, one can perhaps be forgiven for looking backward, and I will indulge. But what I really want to accomplish is to use the past as a point of reference with which to look forward and to explain from my personal viewpoint why, despite three decades of molecular research already in hand—often rife with controversy and contention—I feel the Golgi should continue to hold the attention of molecular cell biologists. To be entirely candid, I am an accidental Golgi researcher, but I will confess in this brief review that I have become an addict. I will recount how the Golgi and I intersected, why it has been a love-hate relationship, and why despite this we are still together.

PRIMER ON THE GOLGI

The Golgi apparatus is a dynamic organelle responsible for receiving, processing, and sorting newly synthesized proteins and lipids through the secretory pathway. In animal cells, it typically consists of four to six flattened micron-sized membrane-bound cisternae arranged as a stack with surrounding vesicles and fenestrated margins that can take the form of tubules and tubular networks. Much has been learned in the past two or three decades about the molecular machinery that contributes to Golgi function. For example, we have a detailed biochemical and structural understanding of how COPI vesicles bud from Golgi cisternae (Lee and Goldberg, 2010), how SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) mediate specific membrane fusion (McNew et al., 2000), how BAR and related proteins create tubular networks (McMahon and Gallop, 2005), how rab proteins create functional domains within a single membrane (Del Conte-Zerial et al., 2008), and how vesicles are tethered at target membranes (Hughson and Reinisch, 2010).

Gradients in composition result in functional specialization of the cisternae within the stack. For example, the glycosyltransferases that process transported glycoproteins and glycolipids are arranged in the order of their enzymatic action, with the first steps taking place in earlier (cis) cisternae (at which proteins arrive from the ER) and the last steps at later (trans) cisternae (where cargo departs for plasma membrane and other post-Golgi destinations). Yet, these same transferases are mobile along the cis–trans axis and even venture back to the ER periodically (Emr et al., 2009). These kinds of gradients, whether of processing enzymes or transport machinery like SNAREs, are therefore the result, at least in part, of a dynamic equilibrium achieved through a balance of “anterograde” (cis→trans) and “retrograde” (trans→cis and trans→ER) flow.

Although it is clear that retrograde flow is mediated by COPI vesicles, there is no clarity on the nature or mix of the.
transport mechanisms that result in anterograde flow (Emr et al., 2009). Two diametrically alternative models have been proposed. The *cisternal progression/mutation model* posits that newest cisterna forms de novo (at the *cis* face) from ER-derived COPII vesicles, while simultaneously the oldest cisterna (at the *trans* face) completely disassembles and disperses as post-Golgi transport intermediates (vesicles and/or tubules). This would create a continuous movement of intact cisternae from *cis* to *trans*, carrying secretory cargo within them without any mechanism for forward transfer between cisternae. To retain Golgi residents (transferases, SNAREs, and the like) and to prevent them from leaving the Golgi with the *trans*-most cisterna, COPI vesicles are proposed to selectively carry such residents in the retrograde direction. By contrast, the *vesicle transport model* posits that the cisternae in the stack are entirely static and that both retrograde and anterograde flow are mediated by budding and fusing COPI vesicles. A recent variant on the vesicle transport model is the *tubule transport model* in which the cisternae are static and budding and fusing membrane tubules are responsible for anterograde transport and COPI vesicles exclusively carry out retrograde transport (Emr et al., 2009).

Broadly speaking, there is solid evidence consistent with each of these mechanisms of anterograde transport, and the emerging consensus is that all three mechanisms likely operate, each serving unique physiological needs (Emr et al., 2009). For example, cargo such as collagen, which naturally aggregates and is too large to fit in a COPI vesicle, are retained within cisternae and transit by cisternal progress. Also tubules may mainly kick in when the cargo load of the Golgi is high, as a kind of back-up mechanism.

CLOSE ENCOUNTERS WITH THE GOLGI

Like most of us, I first heard about the Golgi apparatus in an introductory biology course, and in the 1960s it was still so poorly understood that even good professors at Yale seemed to be unable to convey the most basic concept of what it was good for. I next heard about the Golgi from my Ph.D. thesis advisor, Eugene Kennedy (a person not unfamiliar with organelle biochemistry, having discovered key processes that occur in mitochondria and ER), who conveyed that the Golgi was probably important for something, and someday someone (by implication not him) should get around to looking into it.

In fact, by the late 1960s, when I was at Yale College, James Jamieson and George Palade had concluded that secretory proteins pass through the Golgi apparatus (Jamieson and Palade, 1967), and C. F. Leblond (Neutra and Leblond, 1966) and S. Fleischer (Fleischer et al., 1969) found that complex saccharides are added there, so it was known that the Golgi was a way station for glycoprotein synthesis. It was also clear from Alex Novikoff (Holtzman et al., 1967) and Marilyn Farquhar’s (Friend and Farquhar, 1967) classic histochemistry that the stack is asymmetric with distinct *cis* and *trans* faces and that cisterna progress across the stack (embodied in the then-contemporaneous terminology of “forming face” and “mature face”).

But it was not apparent, at least to me, why cells went to all of this trouble. In other words, why do all eukaryotic cells have a Golgi? Many suggested that it was to allow elaborate saccharide structures to be synthesized for glycoproteins and extracellular matrices and the like. I never found this a compelling argument, because bacteria also make very complex saccharide-based cell walls yet retain the responsible enzymes in a single membrane compartment, so why couldn’t eukaryotes do the same in the ER and discard the Golgi?

It seemed to me, and still does, that there must be a deeper explanation for this organelle, a core concept we were still missing to explain it. My attempt to provide such an underlying structure-function concept to explain the need for this organelle and its stack was the “distillation hypothesis” (Rothman, 1981), which suggested that the cisternae provide a series of chambers allowing progressive purification of plasma membrane (and other) components (which are transported forward) from ER and other components (which are transported backward, a process then unknown but predicted by the model). The distillation hypothesis was highly controversial for various reasons, but over time (now termed “iterative sorting”; Pelham and Rothman, 2000) it has survived and is implicit as a functional outcome common to all models of Golgi function (Emr et al., 2009).

In 1980, Erik Fries and I succeeded in reconstituting vesicle transport in cell-free extracts of tissue culture cells (Fries and Rothman, 1980). The assay we used measured saccharide synthesis as an indirect measure of movement of a viral-encoded membrane glycoprotein (VSV G protein) from glycosyltransferase-deficient membranes of one (mutant “donor”) cell type to wild-type Golgi (“acceptor”) membranes from another cell type in mixed extracts. We had set out to reconstitute ER-to-Golgi transport, radiolabeling the viral protein in the ER before disrupting the donor (mutant) cell, and we tentatively interpreted the reaction as reconstitution of transport from ER to Golgi because the intermediate compartment (ERGIC) remained to be discovered (Schweizer et al., 1991) and transport across the stack was then assumed to be due to cisternal progression, so there seemed to be no way for proteins to move from one stack to another.

We soon found out that the labeled protein had moved beyond the ER and into the Golgi during the time required to harvest and lyse the cells (Fries and Rothman, 1981), and it appeared to us that we were in the odd position of having reconstituted a process—vesicle traffic in the Golgi—that was not actually known to exist! Later work, especially with William Balch, established that vesicles indeed were forming and fusing in the cell-free extracts, and that they both originate and terminate in Golgi stacks (Balch et al., 1984a,b). Prompted by the striking effects of in vivo pulse-chase protocols on the results seen in extracts, with William Dunphy we discovered that Kornfeld’s glycosylation pathway is compartmentalized within the Golgi stack in the *cis-* *trans* direction (Dunphy et al., 1981, 1985; Dunphy and Rothman, 1983), all of which furthered our emerging view that in the cell-free system vesicles carried the viral glycoprotein in the *cis-* *trans* direction.

EMBRACING THE GOLGI

Clearly, it was no longer possible to avoid the Golgi, and the only option was to embrace it. We had to learn more about what went on in the Golgi in order to convincingly interpret our cell-free assay, and this started a long and arduous journey that ultimately impacted cell biology broadly by revealing between 1986 and 1993 the core components of the vesicle budding and membrane fusion of eukaryotic cells.

Purification of cytosolic components required for the cell-free transport reaction yielded NSF with Benjamin Glick, Marc Block, Vivek Malhotra, Lelio Orci, and Felix Wieland (Glick and Rothman, 1987; Block et al., 1988; Malhotra et al., 1988), soluble NSF attachment proteins (SNAPs) with Douglas Clary (Clary et al., 1990), and the SNAREs with Thomas
Sollner (Sollner et al., 1993). Other fruits from this approach included the discovery of COP (now COPI)-coated vesicles with Lelio Orci and Benjamin Glick (Orci et al., 1986), their purification with Vivek Malhotra and Tito Serafini (Malhotra et al., 1989), the identification of their coat protein, coatomer, with Gerard Waters (Waters et al., 1991), and the general GTPase switch mechanism involving the ARF (ADP ribosylation factor) family to trigger coat assembly and later uncoating for fusion with Tito Serafini and Lelio Orci (Serafini et al., 1991).

During this time, with Lelio Orci, we established that the COPI vesicles carry the viral protein between stacks and the fuse (Orci et al., 1989). From all of this we reasonably concluded—prematurely as it turned out—that COPI vesicles only mediate anterograde transport. Matters soon got more complex, starting with Hugh Pelham’s discovery of the first lys-asp-glu-leu (KDEL) signal for retention in the ER, then the KDEL receptor, and ultimately the process of retrograde transport from Golgi to ER (Munro and Pelham, 1987; Sørensen et al., 1990), and then especially when Pierre Cosson and Francois Letourneur discovered that retrograde traffic is carried by COPI vesicles (Cosson and Letourneur, 1994; Letourneur et al., 1994). How could the same vesicle coat carry one set of proteins forward and another backward? This called into question our conclusion that COPI vesicles carry anterograde traffic or at least suggested that there was more to the story. Soon enough, Lelio Orci found that the Golgi contains compositionally distinct subtypes of COPI vesicles: one containing anterograde cargo and the other containing retrograde cargo (Orci et al., 1997), but it was impossible to formally establish that they move in opposite directions. As Palade once famously said, “vesicles do not progress and away from vesicles (for anterograde transport). In the absence of definitive proof, the level of vitriol on all sides began to become unacceptable, and Hugh Pelham and I thought that a review with a balanced perspective could help. We suggested that the available evidence, taken as a whole, implied that both of the proposed mechanisms for anterograde transport—COPI vesicles and cisternal progression—likely operate simultaneously, serving different physiological roles (Pelham and Rothman, 2000). In the decade since then, some recent evidence (Patterson et al., 2008) speaks against progression in the stack for diffusible proteins (as distinct from aggregates like collagen) and points to COPI vesicles, or possibly tubules (Nakano and Luini, 2010), as anterograde carriers.

THE ROAD AHEAD

It seems to me that the field has been largely stalled out for the past decade, largely because truly resolving the central issues evident by the year 2000 required techniques that have not existed. As a result it is understandable that more people have left the field than have entered. Recently, in Barcelona, a representative group of what could fairly be described as good-spirited but battle-weary long-term Golgi researchers (survivors?) came together to assess the situation, thanks to Vivek Malhotra, Suzanne Pfeffer, and Graham Warren. We quickly found each other open-minded to the idea of multiple transport mechanisms in the stack and that we shared optimism that, with newly developing dynamic imaging technologies, answers would be forthcoming in this decade that had eluded us in the past one (Emr et al., 2009).

So in light of this long and complex history, why should anyone still be interested in the Golgi? Why am I energetically returning to the field, and why would I recommend young cell biologists to enter it? In spite of our increasingly detailed molecular understanding, I believe that somehow the “forest may have been missed for the trees” when it comes to the Golgi. We still do not have an integrated understanding—a systems view at the organelle level—of how underlying molecular processes and their specificities combine to explain the overall behavior and function of the organelle as an intermediate biogenesis/transport/processing station.

I believe that our understanding of the basis of anterograde and retrograde transport will rapidly advance when we can clearly establish which genes/proteins/molecular machinery are required for which process. For example, in progression/maturation, fusion proteins such as SNAREs are needed for retrograde flow but not for anterograde transport. If mono-disperse cargo (albumen, VSV G) moves anterogradely by COPI vesicles and collagen moves by progression/maturation, fusion proteins such as SNAREs and COPI components, and the latter will not. On the other hand, if tubules mediate anterograde transport, the tabulation proteins and SNAREs will be required. And so on.

It seems like such a simple and obvious thing to do, so why hasn’t it happened? Why has Golgi research failed for so long to deliver a definitive view on such fundamentals? Naively, if we could simply watch the dynamics of individual proteins (cargo, coats, SNAREs, etc.)—where they are one moment and where they are the next—these kinds of basic questions would be quickly answered! In reality, we have the resolution in the electron microscope, but unfortunately the images are by nature static. We have the time resolution with green fluorescent protein (GFP)-tagged proteins/fluorescence microscopy, but unfortunately the spatial resolution (~250 nm) is currently inadequate (~50 nm will...
be needed, about the diameter of typical vesicles/tubules). The spatial resolution problem is at the light level is compounded by the fact that the cisternae are closely stacked, their center-to-center separation (~100 nm) being near but unfortunately below optical resolution.

My view is that Golgi researchers can profitably take some lessons from the pioneering work of Marino Zerial (Sonnichsen et al., 2000) with endosomes where enormous progress has been made in the past decade in the same technology landscape in which Golgi research has lagged behind. Like the Golgi, endosomes consist of multiple compartments linked via anterograde and retrograde transport, whose compositional differences are maintained in the face of bidirectional flow. The main morphological difference is that endosomal compartments are not stacked. Endosomal compartments can be many microns apart, and their locations evolve with time. This has allowed compositional and morphological changes (such as rab-conversion, homotypic fusion, and tubule formation) of individual endosomal compartments to be visualized and quantified during a single round of transport, which is not possible when compartments are closely stacked as in the Golgi. The importance of spatial separation is underscored by the recent success in studies of the naturally unstacked Golgi cisternae of the yeast *Saccharomyces cerevisiae* by Glick (Losev et al., 2006) and Nakano (Matsuura-Tokita et al., 2006), which suggested that maturation plays a major role here.

In addition, Golgi research has been confounded by the lack of methods for rapidly inhibiting specific molecular machinery. Most current studies of trafficking in animal cells employ RNA interference (RNAi) to chronically reduce the level of a targeted piece of protein machinery. Whereas such studies can show that a gene/protein/molecular mechanism is broadly required for Golgi function, they cannot reveal whether that requirement applies to anterograde or retrograde movement or both. Chronic depletion results in terminal phenotypes that are typically complex and confusing if not impossible to interpret because there are likely to be feedback mechanisms that maintain a balance between anterograde and retrograde flow, a core element of any model that yields a stable dynamic equilibrium. Chronically inhibiting transport in one direction should eventually suppress transport in the other direction. An old but still powerful example of this is the sec21 mutant in yeast, which accumulates secretory proteins in the ER (Novick et al., 1980). Yet, Sec21 protein is a subunit of the COPI coat (Stenbeck et al., 1992). On the face of it, one might conclude from this alone that COPI mediates transport from ER to Golgi. But of course the opposite is the case!

To resolve such profound ambiguities, it is necessary to rapidly inactivate the function of a targeted protein/gene within the time frame of a single round of Golgi transport (5–30 min in animal cells). Obviously this must be done at the protein rather than the gene expression level. This is easily done in cell-free systems by adding antibodies or controlling time of incubation in the absence of a single protein, but is much more difficult in living cells. I believe that success in this technical arena will open many doors. Rapid inactivation should ideally also be rapidly reversible to show that morphology and dynamics return (i.e., pathology has not been introduced) and to establish that any morphological structures accumulating (whether at light or electron microscopy levels) are consumed after reversal and thus are bona fide intermediates.

### SUMMARY

This admittedly and by intent personal perspective on the field suggests that there are two key requirements needed to establish the molecular requirements for anterograde as distinct from retrograde transport in the Golgi:

1. Methods to visualize and establish the sequential presence of tagged proteins in donor and acceptor compartments that are separated from each other sufficiently for clear optical resolution in living cells. This will allow one round of transport to be observed. Old approaches to this that are still available include transfer of proteins between distinguishable Golgi in fused cells (Rothman et al., 1984). Newer and ultimately much more versatile and systematic approaches are based on super-resolution imaging (Schermelleh et al., 2010). These technologies hold great promise, but need better time resolution (photocatalyzed localization microscopy [PALM]) and imaging duration (stimulated emission depletion [STED]) before they will see their full impact. Golgi research will progress in lock-step with the new technologies for dynamic visualization with molecular specificity.

2. Methods to specifically and reversibly inactivate the function in the Golgi of a targeted protein. Examples of this kind of technology include the controlled and reversible release of functional proteins from aggregates based on ligand-induced oligomerization (Spencer et al., 1993) and the use of photosensitive GFP-tagged proteins, such as “killer red” (Bulina et al., 2006), to burn away the tagged protein at a given time and place. Both of these technologies are facilitated by RNAi to reduce or eliminate endogenous copies of the targeted protein on a chronic basis, whereas a second, RNAi-resistant tagged-copy is subject to temporal control.

### ACKNOWLEDGMENTS

I acknowledge the many fine students, postdoctoral fellows, and staff whom it has been my privilege to work with and who have contributed so much over the years, both when in my lab and more importantly afterward. I also gratefully acknowledge continuous support from the National Institutes of Health, which has enabled all of this.

### REFERENCES


