

Journeys through the Golgi—taking stock in a new era

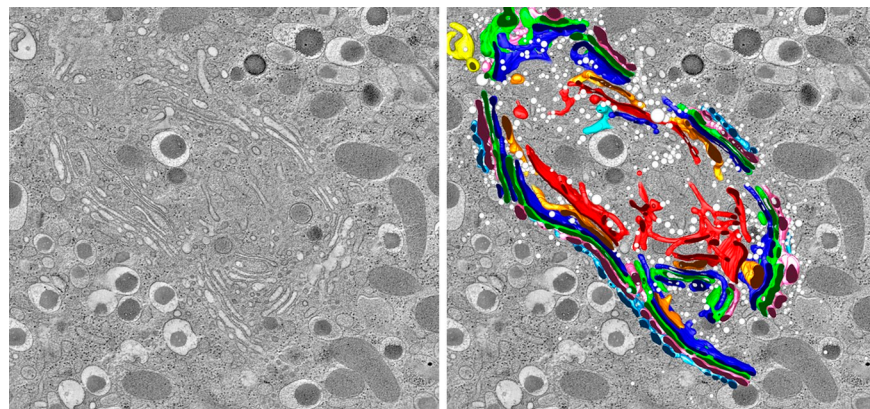
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The Golgi apparatus is essential for protein sorting and transport. Many researchers have long been fascinated with the form and function of this organelle. Yet, despite decades of scrutiny, the mechanisms by which proteins are transported across the Golgi remain controversial. At a recent meeting, many prominent Golgi researchers assembled to critically evaluate the core issues in the field. This report presents the outcome of their discussions and highlights the key open questions that will help guide the field into a new era.

Introduction

Until about ten years ago, the Golgi was viewed as a stable, independent structure that exchanges components with other organelles. Then the pendulum swung in the other direction, and the Golgi was more frequently viewed as a dynamic structure that constantly regenerates by the self-organization of components supplied by other organelles. This has been a hotly debated issue that has divided the field for a long time. Now it seems likely that the truth lies somewhere in between. This field has been marked by vocal controversies that have made the subject seem complex, confusing, and even unwelcoming to outsiders. Why has there been so much debate about the Golgi and why should anyone care? The major reason is the essential role of Golgi membranes in sorting and transport of proteins; an understanding of how the Golgi is made and how it works is therefore of



A tomographic image slice (left) from a 3D reconstruction (right) of a section of insulin-secreting beta pancreatic mouse cell shows the “stacks” of Golgi cisternae distributed along the length of the ribbon. The clathrin-coated trans-most cisterna (red) or “TGN” at the center of the ribbon reveals the extraordinary complexity of Golgi architecture. Golgi cisternae (cis-trans): light blue, pink, green, dark blue, gold, red; Golgi-associated vesicles: white.

fundamental importance. Unfortunately, the available tools have not been adequate to answer certain key questions directly and forcefully. This situation is improving as new technologies enable us to revisit old topics. For example, electron tomography has revolutionized the analysis of Golgi structure (Marsh, 2005), and organellar proteomics are defining membrane compartments in unprecedented detail (Gilchrist et al., 2006). A particularly exciting development is super-resolution microscopy, which permits live-cell imaging at a spatial resolution that has previously been the realm of electron microscopy (Hell, 2009; Lippincott-Schwartz and Manley, 2009). As these and other techniques blossom during the coming years, we will enter a new era in Golgi research.

Some features of the Golgi are already well understood. Newly synthesized proteins move from the ER to the

Golgi, where they are processed before being sorted to their ultimate destinations (Mellman and Warren, 2000). This processing includes carbohydrate modifications and proteolytic cleavage events. The Golgi is often composed of disk-like membranes called cisternae, and early- and late-acting processing enzymes are concentrated in distinct cisternae (Dunphy and Rothman, 1985). Golgi cisternae in higher eukaryotes are arranged in ordered stacks of 4–8 cisternae. Newly synthesized proteins arrive at the cis side of a Golgi stack and then travel across the stack to the opposite, trans side before being exported from the trans-Golgi network (TGN) (Griffiths and Simons,

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1986). This arrangement allows for iterative sorting of both secretory cargo proteins and resident Golgi and ER proteins (Rothman, 1981). COPII-coated vesicles carry newly synthesized proteins from the ER to the Golgi (Lee et al., 2004). It is agreed that COPI-coated vesicles retrieve components from the Golgi to the ER, and also transport material between Golgi cisternae, but their involvement in forward movement of cargo across the Golgi is unclear (Orci et al., 2000; Cosson et al., 2002; Rabouille and Klumperman, 2005).

To look beyond these generally accepted findings, a group of Golgi researchers gathered in June, 2009, at the Center for Genomic Regulation (CRG) in Barcelona, with funding support from the Catalan Institution for Research and Advanced Studies (ICREA). Our goal was to discuss current ideas about the Golgi, critically evaluate the strengths and limitations of published data, and try to convey the excitement of this field in a manner that will attract new participants. As summarized below, the discussion focused on four key questions.

1. How are proteins transported through the Golgi?

The discovery that COPI vesicles carry retrograde traffic, such as the KDEL receptor, from Golgi to ER (Martinez-Menárguez et al., 2001; Cosson et al., 2002) led to the widespread assumption that the only role for COPI was as a retrograde carrier. More recent studies have revealed that animal cells contain at least three distinct isoforms of coatamers that appear to assemble separately and regionally within the Golgi (Moelleken et al., 2007). Although it is not clear whether these coatamer isoforms localize to vesicles containing different cargo, these findings revive the possibility that COPI may carry traffic in both the anterograde and retrograde direction within a stack. While the role of COPI vesicles in forward transport awaits further analysis, the cisternal maturation mode of cargo transport is now the favored model (Glick and Malhotra, 1998). This model assumes that Golgi cisternae form de novo, progressively mature, and ultimately

dissipate. In a stacked Golgi, new cisternae would form at the cis face, progress through the stack, and peel off from the trans face (Mollenhauer and Morré, 1991). Secretory cargo proteins are thought to be carried forward by this process of cisternal progression. Meanwhile, the progressing cisternae would mature by the recycling of resident Golgi proteins from older to younger cisternae.

Evidence in support for cisternal progression came from studies of mammalian procollagen I, which folds in the ER into rod-like trimers that further assemble in the Golgi into large (~300 x 150 nm), stable, cylindrical aggregates (Leblond, 1989; Beck et al., 1996). Procollagen can be accumulated in the ER and then released in a synchronized “wave” for transport through the Golgi. This approach was combined with 3D electron microscopy to demonstrate that procollagen aggregates traverse the Golgi without ever leaving the lumen of the cisternae (Bonfanti, et al., 1998). Thus, procollagen apparently moves through the Golgi by the progression of



Golgi researchers gathered in June, 2009, in Barcelona. From left to right: Scott Emr, Catherine Rabouille, Alberto Luini, Ben Glick, Brad Marsh, Jennifer Lippincott-Schwartz, Felix Wieland, Graham Warren, Suzanne Pfeffer, Jim Rothman, Aki Nakano, Adam Linstedt, and Vivek Malhotra.

cisternae from the cis to the trans side of the stack. Similar conclusions have been reached for large secretory cargoes in other cell types, e.g., scales in algae (Becker et al., 1995). It is still uncertain whether smaller secretory cargoes follow the same pathway. Nevertheless, these findings support one of the key assumptions of cisternal maturation, namely that Golgi cisternae can act as forward carriers for secretory cargo transport.

Cisternal maturation was directly visualized using the yeast *Saccharomyces cerevisiae*, in which individual Golgi cisternae are dispersed throughout the cytoplasm and are therefore optically resolvable by fluorescence microscopy (Wooding and Pelham, 1998). Fluorescent protein tags were used to label early and late Golgi cisternae green and red, respectively. The cisternal maturation model predicted that a green fluorescent spot should become visible as a cisterna formed, then turn red as the cisterna matured, then lose all fluorescence. This prediction was confirmed by confocal microscopy (Losev et al., 2006; Matsuura-Tokita et al., 2006).

Because membrane traffic mechanisms are generally conserved, cisternal maturation may emerge as a general principle of Golgi function. However, the evidence is still incomplete. Most notably, the two key assumptions of the cisternal maturation model have been verified in different cell types: mammalian cells revealed that Golgi cisternae act as forward carriers for secretory cargoes, and yeast cells revealed that Golgi cisternae mature. A future goal will be to test both assumptions in each of these cell types. Super-resolution microscopy may permit the tracking of individual cisternae in the stacked mammalian Golgi, while three-color video microscopy of yeast should permit secretory cargoes to be visualized within the maturing cisternae.

The mechanism of Golgi maturation is still uncertain. Transmembrane Golgi resident proteins move between cisternae (Losev et al., 2006; Matsuura-Tokita et al., 2006), implying the existence of either dissociative carriers or membrane continuities. The best candidates for dissociative carriers are COPI vesicles. However, the contents and directionality of COPI vesicles are not yet clear. Different

researchers have reached divergent conclusions about whether mammalian COPI vesicles contain resident Golgi proteins and/or secretory cargo proteins (Orci et al., 2000; Cosson et al., 2002; Rabouille and Klumperman, 2005; Gilchrist et al., 2006). Data from *S. cerevisiae* mutants are also ambiguous because in yeast strains carrying temperature-sensitive mutations in COPI subunits, Golgi maturation is slowed but not arrested (Matsuura-Tokita et al., 2006), and certain secretory cargoes can still be secreted (Gaynor and Emr, 1997). A possible way to reconcile some of these observations is to postulate, as mentioned above, that COPI vesicles travel in both anterograde and retrograde directions. Mechanistic analysis of COPI-mediated transport will be a goal for the coming years.

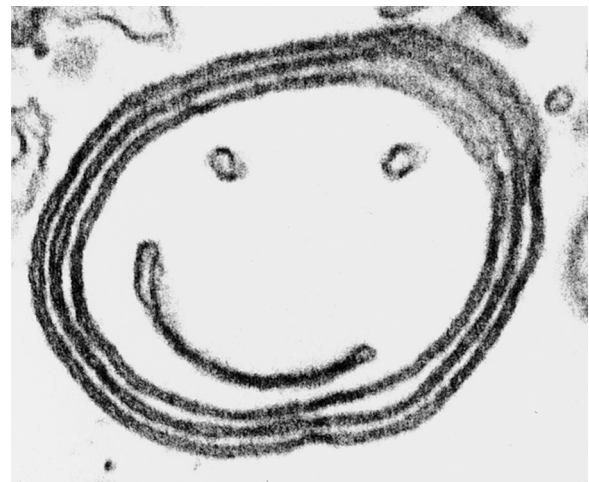
Another uncertainty is the status of the TGN. One interpretation views the TGN as a transient Golgi compartment that ultimately matures into secretory vesicles (Glick and Malhotra, 1998). However, the TGN is distinct from earlier Golgi cisternae with regard to morphology, domain organization, protein composition, localization signals for resident proteins, and transport carrier production (Bard and Malhotra, 2006). In some respects, the TGN is more similar to endosomes than to earlier Golgi cisternae (Glick and Nakano, 2009). These observations suggest that the TGN might display unique dynamics. For example, terminally mature trans-Golgi cisternae might fuse with a long-lived TGN, which would accumulate and concentrate secretory cargoes. More generally, one can speculate that the secretory pathway includes a mixture of long-lived and transient compartments (Appenzeller-Herzog and Hauri, 2006).

2. What is the significance of connections between Golgi cisternae?

Golgi cisternae have generally been thought to be physically and functionally distinct (Dunphy and

Rothman, 1985), but accumulating evidence suggests that cisternae can sometimes be interconnected by membrane tubules. These studies have focused on the mammalian Golgi. It has long been known that mammalian Golgi stacks are connected laterally by membrane tubules to form a “Golgi ribbon” (Rambourg and Clermont, 1990). More recently, electron tomography has revealed that different cisternae can be connected within an individual stack (Marsh et al., 2004; Trucco et al., 2004). These heterologous connections seem to be more prevalent when cells are experiencing high levels of Golgi traffic, but they can be observed in cells grown under standard conditions (Bouchet-Marquis et al., 2008; Vivero-Salmerón et al., 2008). The frequency and duration of heterologous tubular connections is still being debated. An obvious concern is the choice of experimental models used in some of these studies as well as technical issues related to how cells are processed before imaging. It will be important to elucidate the mechanisms that control membrane connectivity, and to standardize methods for capturing cellular compartments in an unperturbed state.

In mechanistic terms, the challenge is to evaluate the importance of heterologous tubular connections between cisternae. Such tubules have the potential to mediate rapid passage of small soluble proteins across the Golgi stack. Candidate proteins for this putative “fast track” pathway include albumin and proinsulin. Both proteins are nonglycosylated, and therefore do not require prolonged exposure to



In vitro reconstituted *Xenopus* Golgi membranes.

processing enzymes. Perhaps heterologous tubular connections allow cells to secrete such proteins in large amounts without moving large amounts of membrane through the Golgi. Models of this type raise the question as to how the Golgi stack maintains its biochemical polarity when different compartments are interconnected. A more general question is whether heterologous tubular connections are an essential, conserved aspect of Golgi function, or whether they are a specialization of mammalian cells.

3. What is the role of membrane domains within the Golgi?

It was recently postulated that the Golgi apparatus is a continuous two-phase partitioning system, with one phase enriched in processing enzymes and the other phase in export machinery (Patterson et al., 2008). After secretory cargo proteins enter the Golgi, they would partition rapidly in and out of the processing domains while being exported in a stochastic manner from the export domains. This concept was developed to explain the surprising observation that secretory cargoes exit the Golgi with exponential kinetics, not with the linear kinetics that would be expected from cisternal progression/maturation. However, in solving the problem of exit kinetics, the rapid partitioning model discards other well-established observations such as sequential glycosylation: in all eukaryotes examined to date, early-acting Golgi enzymes are concentrated in cis cisternae whereas late-acting Golgi enzymes are concentrated in trans cisternae, implying that secretory cargo proteins move vectorially through the Golgi while being sequentially processed (Dunphy and Rothman, 1985). Indeed, waves of cargo have been observed moving across mammalian Golgi stacks (Trucco et al., 2004). Such findings are at odds with the rapid partitioning model, which posits the exit of secretory cargo proteins at all levels of the Golgi. This in turn could result in export of incompletely processed secretory cargo from the Golgi, something that does not appear to happen under normal physiological conditions.

As an alternative to rapid partitioning, can the cisternal maturation model be revised to accommodate exponential

Future Directions in Golgi Research

1. What cargoes do COPI vesicles carry, how many types of COPI vesicles are there, and in which directions do these various vesicles travel?
2. Do different secretory cargoes follow distinct routes through the Golgi?
3. What molecular mechanisms drive and regulate cisternal maturation?
4. What are the functions of tubular connections between heterologous cisternae, and how do these tubules form? Is tubule formation mechanistically related to vesicle formation?
5. Are there specialized domains in Golgi cisternae? How are they created, and what roles do they play in cargo sorting and export?
6. How are Golgi compartments constructed and remodeled?
7. Is Golgi stacking fundamentally important for membrane traffic? If so, how do organisms such as *S. cerevisiae* bypass this requirement?

kinetics of cargo exit from the Golgi? One possibility, as described above, is that the TGN is a relatively stable compartment. Secretory cargo proteins might be delivered from transient Golgi cisternae to a long-lived TGN, from which they would depart with first-order kinetics. This possibility was considered by Patterson et al. (2008) but was not convincingly excluded. The stable TGN model predicts that secretory cargo proteins should be concentrated in the TGN relative to Golgi cisternae. Experimental tests of this idea will refine our understanding of traffic through the secretory pathway.

4. Is the Golgi an independent organelle?

The Golgi is remarkably dynamic. For example, the drug brefeldin A (BFA) causes the mammalian Golgi to fuse with the ER, but subsequent removal of the drug allows the Golgi to reappear. In both mammalian cells and fungi, entire Golgi stacks seem to be capable of forming de novo (Bevis et al., 2002; Puri and Linstedt, 2003). This idea has been extended by proposing that the ER is an intermediate in the reorganization of the mammalian Golgi during mitosis or upon microtubule disruption (Zaal et al., 1999). However, the weight of the evidence now indicates that the Golgi has considerable autonomy. For example, many Golgi proteins only rarely cycle through the ER (Jesch and Linstedt, 1998; Jokitalo et al., 2001; Pecot and Malhotra, 2004, 2006; Rhee et al., 2005), and multiple groups (Graham Warren, Vivek Malhotra, Adam Linstedt,

Joachim Seemann, and John Lucocq) have found that Golgi proteins remain separate from the ER during mammalian mitosis. Although the mechanisms that organize and reorganize the Golgi are still mysterious, the published data suggest that once a Golgi apparatus has formed, it can be remodeled independently of the ER.

Conclusions

The purpose of this meeting was to critically evaluate published data on the specific topics listed in this meeting review. There was extensive support for the cisternal maturation mode of transport and for the existence of tubules connecting Golgi cisternae in a stack. Furthermore, it was concluded that the Golgi membranes are maintained independent of the ER under normal physiological conditions. However, after considerable deliberations it was concluded that the contents of COPI vesicles and their directionality in the secretory pathway remains unclear. We predict that advances in high-resolution microscopy and biochemical techniques will help to clarify this issue. Finally, these discussions resulted in a list of open questions about the Golgi (see Future Directions in Golgi Research box) that we think warrant further exploration. We hope that our dialogue will help drive the field forward and keep researchers engaged with the Golgi apparatus for years to come.

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