Title

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Journal

Trends in Cell Biology, 8(1)

ISSN

0962-8924

Authors

Farquhar, Marilyn G

Palade, George E

Publication Date

1998

DOI

10.1016/s0962-8924(97)01187-2

Peer reviewed

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The Golgi apparatus: 100 years of progress and controversy

Marilyn G. Farquhar and George E. Palade

Research on the Golgi apparatus has resulted in major advances in understanding its structure and functions, but many important questions remain unanswered. The history of the Golgi apparatus has been marked by arguments and controversies, some of which have been resolved, whereas others are still ongoing. This article charts progress in understanding the role of the Golgi apparatus during the 100 years since it was discovered, highlighting major milestones and discoveries that have led to the concepts of the organization and functions of this organelle that we have today.

While studying nerve cells stained by the metal-impregnation technique that now bears his name, Camillo Golgi noticed a basket-like network surrounding the nucleus in Purkinje cells (Fig. 1). Thus the Golgi apparatus was discovered. Golgi published the first description of this structure in 1898, calling it the 'apparato reticolare interno' or 'internal reticular apparatus'[1]. The name was shortened to 'Golgi apparatus' or 'Golgi complex' and often, recently, to just 'the Golgi'.

The original controversy: Is the Golgi apparatus an artifact?

The striking feature of the history of the Golgi is that it has been fraught with controversies – and still is. To begin with, it was hotly debated for over 50 years whether the Golgi was a bona fide organelle or a gross artifact. The debate raged because the Golgi was not visible in living cells and its visualization depended on Golgi's capricious heavy-metal staining method, called the black reaction (la reazione nera), which was difficult to reproduce reliably and stained many other structures, including whole neurons. At the time he discovered the Golgi apparatus, Golgi himself was involved in a debate (which he eventually lost) with Ramón y Cajal over whether neurons were discontinuous or formed a continuous network. Nevertheless, in 1906, he shared the Nobel prize for Physiology and Medicine with Cajal, not for the discovery of the Golgi apparatus but for the introduction of the black reaction to the study of the nervous system, which was used by Cajal to prove him wrong. Ironically, to this very day, a modified form of Golgi's staining procedure is used by neuronal cell biologists for defining individual neurons and tracing neuronal networks.

How was the controversy over the very existence of the Golgi apparatus put to rest? As with many such controversies in the era of light-microscopic cytology, it was resolved only with the introduction of the electron microscope and its application to the study of cell structure. In the first descriptions of the Golgi at the electron-microscopic (EM) level by Dalton and Felix[2] and Sjostrand and Hanzon[3], a stack of curved, smooth-surfaced cisternae (then called lamellae) surrounded by vacuoles of variable size (Fig. 2) was seen in the regions of cells where the Golgi apparatus was detected by Golgi staining with light microscopy. An avalanche of similar observations during the 1950s established the generality of these structures and validated the ubiquitous existence of the Golgi apparatus and its inherent variability and complexity.

From that time on, the Golgi apparatus became a centre of great attention and excitement in cell biology. Here, we outline some of the important milestones in Golgi research of the past four decades (Table 1) and some of the controversies that have arisen along the way.

The 1960s: delineation of functions

Until the 1960s, there was abundant speculation but little direct information on the functions of the Golgi apparatus. It had long been recognized by light microscopists that the Golgi was highly developed in secretory cells, but not until the 1960s did the role of the Golgi in secretion and glycosylation become clear. Once again, it was the introduction of new techniques, in this case cell fractionation and EM autoradiography, that was crucial to this progress. These two approaches applied to the exocrine pancreas, a cell type highly specialized for protein secretion, were used by Palade[4] to obtain complementary biochemical and morphological data delineating the vectorial transport of secretory proteins through the cell, the involvement of the Golgi in this process and the existence of vesicular transport to the Golgi.

Convergent results obtained by cell fractionation and EM autoradiography also established the role of the Golgi in glycosylation. Two landmark sets of findings can be recognized. First, Fleischer *et al.*[5] and Morré *et al.*[6] developed methods for the preparation of Golgi fractions and showed that galactosyltransferase is enriched in them, thus providing a Golgi marker enzyme. Second, Leblond and coworkers[7,8] demonstrated by EM autoradiography the uptake of two sugars, glucose and galactose, into the Golgi. Both sets of findings pointed to a crucial role for the Golgi in glycoprotein synthesis. They also set the stage for the delineation of the Golgi's functions in *N*-linked glycosylation, the division of labour between the endoplasmic reticulum (ER) and Golgi in this process, and the compartmentation of glycosylation reactions in the Golgi, which took place during the 1970s and 1980s. During the same period, autoradiographic findings

The authors are in the Division of Cellular and Molecular Medicine and Depts of Pathology (M. G. F.) and Medicine (G. E. P.), University of California, San Diego, CA, USA. E-mail: mfarquhar@ucsd.edu

 0962-8924/98/$19.00
PII: S0962-8924(97)01187-2

by Godman and Lane[9] demonstrating uptake of sulfate into the Golgi implicated the organelle in sulfation and therefore in the biosynthesis of proteoglycans.

Recognition of distinct Golgi compartments

Another major conceptual development in the 1960s was the realization that the Golgi apparatus consists of distinct subcompartments. This was made possible by the adaptation of lead-phosphate-based enzyme cytochemical methods to the EM level. It was recognized originally by Novikoff and Goldfischer[10] and extended by others[11] that the Golgi enzymes acid phosphatase and thiamine pyrophosphatase are not distributed uniformly across the stack. Instead, they are located on one side, now recognized as the *trans* side of the Golgi stack. Other phosphatases were similarly found to have distinctive localizations in the Golgi stack. A few years later, Friend[12] adapted Golgi's heavy-metal impregnation method for EM and showed, ironically, that the Golgi stack is not stained homogeneously. Only one side, now recognized as the *cis* side, is stained by this method. Interestingly, we still do not understand the precise biochemical basis for deposition of heavy metals in the *cis* Golgi. However, the collective EM findings of all these cytochemical studies gave the first clear evidence of heterogeneity among Golgi elements. Results obtained by enzyme cytochemistry at the EM level also firmly established the role of the Golgi in packaging of lysosomal enzymes for delivery to lysosomes[13,14].

However, this period was not without controversy. The results obtained by enzyme cytochemistry led to a long and heated debate concerning the significance of the localization of acid phosphatase, the main lysosomal marker at the time, in the Golgi. Novikoff *et al.*[15] noted the special morphological features of the cisterna (or 'saccule') on one side of the Golgi where acid phosphatase is localized and proposed the name GERL (Golgi–ER–lysosomes) for this cisterna. The GERL hypothesis held that special regions of ER synthesize lysosomal enzymes, among them acid phosphatase, and channel them directly to the GERL cisterna for delivery to lysosomes. The GERL concept was the subject of many heated discussions between Novikoff and disbelievers that took place at Lysosome Gordon conferences and at the annual meeting of the American Society of Cell Biology over a period of more than 10 years. Eventually, it was proven that lysosomal enzymes follow the same route to and through the Golgi as other glycoproteins. The GERL concept had the virtue that it fixed the attention of the cell-biology world on the special features of the *trans*-most cisterna and paved the way for the conceptualization of the distinctive properties of the *trans*-Golgi network (TGN)[16].

The functional significance of the restricted distribution of enzymes within the Golgi remained a mystery for some time. Delineation of the steps in *N*-glycosylation in the 1970s by Schachter and Kornfeld paved the way for further development and refinement of the concept of Golgi compartments by linkage to steps in *N*-glycosylation. It became evident that enzymes involved in *N*-linked glycosylation are arranged in space as they act over time, with

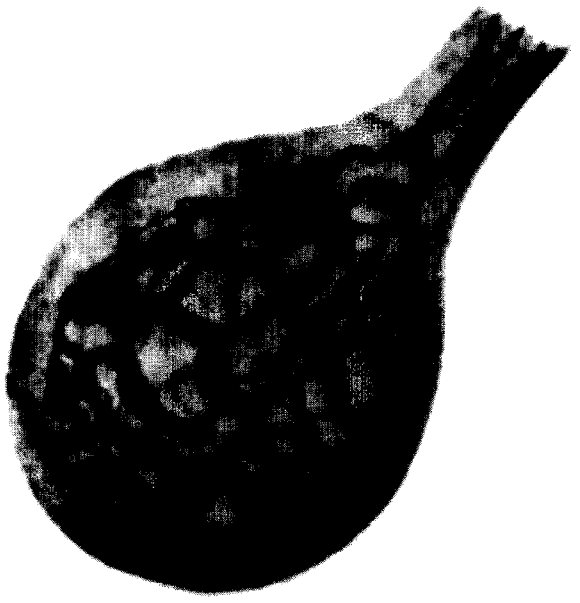

FIGURE 1

What Golgi saw. The first demonstration of the Golgi apparatus as a basket-like network surrounding the nucleus in Purkinje cells stained by metallic impregnation. (Reproduced from Ref. 1.)

early-acting enzymes (α-mannosidase I and GlcNAc phosphotransferase) located in the *cis* Golgi, and late-acting Golgi enzymes (galactosyl- and sialyltransferases) located in *trans* Golgi cisternae (reviewed in Refs 17 and 18). Once again, this conceptual advance became possible as a result of new technical advances. Immunogold labelling was used by Roth and Berger[19,20] to demonstrate that the late-acting Golgi enzymes galactosyl- and sialyltransferase are localized to *trans* cisternae and the TGN. These results could be correlated with the biochemical findings of Dunphy *et al.*[21] and Goldberg and Kornfeld[22] showing that there is a gradual decrease in density of Golgi membranes across the stack that allows their partial separation on sucrose density gradients: galactosyltransferase peaks in light Golgi fractions and GlcNAc phosphotransferase, an early-Golgi enzyme, peaks in heavy Golgi fractions.

The precise number of Golgi compartments is still debated, but most investigators currently recognize four: *cis* (sometimes called the CGN), *medial*, *trans* and *trans*-Golgi network (or TGN; see Fig. 3). Each of these compartments has presumptive markers used as guideposts, but it became evident that the boundaries between these compartments are not distinct. After a period in the 1980s when concepts of Golgi compartments were rather rigid, it became clear from immunocytochemical studies that differences exist among different cell types in the distribution of marker enzymes for Golgi[20,23]. Moreover, biochemical results obtained by freeze-frame analysis of the Golgi validated overlap in Golgi-modifying enzymes[24].

In 1984, Saraste and Kuismanen[25] reported the existence of a novel pre-Golgi compartment located between the transitional ER and the *cis* Golgi where newly synthesized cargo (Semliki Forest virus spike protein) accumulates when cells are incubated at low temperature (15°C). Whether this structure was an artifact of the low temperature incubation or a valid

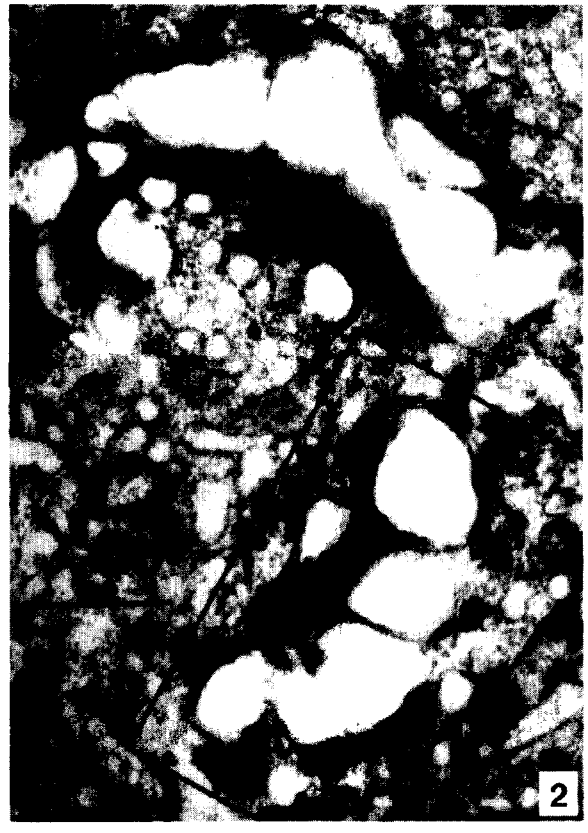

FIGURE 2

First electron micrograph of the Golgi apparatus. The controversy regarding whether Golgi's apparatus was real or an artifact was resolved when the electron microscope revealed a regular structure composed of 'lamellae' and vacuoles at sites of staining with heavy metals. (Reproduced, with permission, from Ref. 2.)

entity was debated initially. Recognition of the distinct morphological and biochemical features of this compartment, now commonly referred to as ERGIC (endoplasmic reticulum–Golgi intermediate compartment) or VTCs (vesicular–tubular clusters), came about as a result of the discovery of the only known marker for this compartment, p53 (Ref. 26), and its rat homologue, p58[27,28], now called ERGIC53/58. ERGIC53/58 is a very intriguing protein that cycles between the ER, ERGIC and the *cis* Golgi and has properties of a mannose-binding lectin, which has stimulated speculation that it might be a sorting lectin for the early secretory pathway[29]. At present, the existence of this pre-Golgi intermediate compartment is accepted, but its boundaries and functions are still debated.

Transport to the Golgi apparatus: bulk flow or selective transport?

Figure 3 shows current concepts of the organization of the Golgi apparatus and of biosynthetic and recycling membrane traffic to and through it that have evolved over the past 30 years. How was the route of general traffic flow determined? The work of Palade on the exocrine pancreas[4] established that secretory proteins move vectorially through the cell from transitional ER elements to the Golgi, where they are packaged into secretory granules that are discharged by exocytosis. This work also established that transport at least from the ER to the Golgi was energy dependent and operated through vesicles. Later on, it was shown that membrane proteins[30] and lysosomal enzymes[31] follow exactly the same route up to the TGN.

Whether transport between the ER and the Golgi is selective or occurs by bulk flow is a topic that has been debated since the 1970s[32–34]. What is the history of this controversy? In 1987, Wieland *et al.*[35] employed a novel approach in which acyl tripeptides were applied to cells to distinguish between the two models – i.e. selective transport and bulk flow. From these experiments, it was concluded that newly synthesized proteins move from the ER to the Golgi and through the Golgi by bulk flow. However, subsequent work was difficult to reconcile with this hypothesis[36,37]. Balch *et al.*[37] provided strong evidence that proteins leaving the ER are sorted for packaging into carrier vesicles rather than being transported by bulk flow. Cargo was shown to undergo concentration (5–10-fold) at the time of exit. Moreover, studies in yeast demonstrated selective sorting of cargo[38]. Quite recently, Nishimura and Balch[39] reported the identification of a di-acidic sorting signal (Asp-x-Glu, or DxE) on the cytoplasmic tail of the VSV-G protein that is required for its efficient recruitment into transport vesicles exiting the ER. The identification of this signal, found on a number of transmembrane proteins, provides further evidence that export from the ER occurs through a selective mechanism. Subsequently, the original work by Rothman and coworkers, which was taken to support the bulk flow model, was revisited by the authors, who concluded that their earlier conclusions were incorrect and should be reinterpreted and taken to support the concept of selective transport[40]. In fact, the collective evidence derived from *in vitro* studies[41] and from studies on yeast mutants[42] provides strong support for the selective-transport model.

Retention and retrieval signals

Once the magnitude and diversity of membrane trafficking was realized, it became evident that the cell must have mechanisms that allow cargo to move down the secretory pathway but retain resident proteins in each organelle. The first insights into the nature of these mechanisms were obtained in 1987 from the results of Machamer and Rose[43] and Munro and Pelham[44] who took advantage of new approaches that became available as a result of the application of recombinant DNA technology to problems in cell biology.

Machamer and Rose obtained the unorthodox and unexpected finding that information on targeting and retention of Golgi membrane proteins resides in the transmembrane domain of Golgi-targeted proteins. Using as a model the E1 glycoprotein of the MHV coronavirus, which buds from the *cis* Golgi, they showed that one of three transmembrane domains of the EI capsid protein (now called M protein) served as a retention signal and was sufficient for targeting of this protein to the Golgi. Work since then has pointed to the transmembrane domain as an important site of targeting information, but the

TABLE 1 – SOME IMPORTANT MILESTONES IN GOLGI RESEARCH

Year	Event	Discoverer(s)
1898	Discovery of the Golgi apparatus	Golgi
1954	First electron microscopy (EM) description of the Golgi apparatus	Dalton and Felix
1957	Cisternal maturation model of Golgi transport	Grassé
1961	Compartmentalization: regional distribution of enzymes	Novikoff and Goldfischer
1964	Involvement in sulfation	Godman and Lane
1966	Involvement in glycosylation: glucose incorporation	Neutra and Leblond
1967–1975	Role in secretory pathway defined and vesicular transport documented	Palade, Jamieson and coworkers
1969	Incorporation of mannose in endoplasmic reticulum (ER), galactose in Golgi	Whur, Herscovics and Leblond
	Galactosyltransferase as a biochemical marker for the Golgi apparatus	B. Fleischer *et al.*; Morré *et al.*
1971	GERL concept	Novikoff and Novikoff
1973–1981	Role of mannose 6-phosphate in lysosomal enzyme sorting by Golgi	Sly, Neufeld, Kornfeld, Jourdian
1977	Demonstration of recycling plasma membrane to Golgi	Herzog and Farquhar
1980	Introduction of glycosidase (endo H) treatment to assess transport	Strous and Lodish
1981–1983	Topology of N-glycosylation	Dunphy and Rothman
	Immunocytochemical localization of galactosyltransferase to *trans* Golgi	Roth and Berger
	Reconstitution *in vitro* of transport within Golgi stack	Rothman *et al.*
1984	Description of 15° block and cargo accumulation in pre-Golgi intermediate compartment	Saraste and Kuismanen
1985	Regulated vs. constitutive secretory pathways	Moore and Kelly
1986	Description of 20° block and cargo accumulation in *trans*-Golgi network (TGN)	Griffiths and Simons
1987	Transmembrane domain required for retention of resident Golgi proteins	Machamer and Rose
	KDEL retrieval signal for resident ER proteins	Munro and Pelham
	Involvement of small GTP-binding proteins in vesicular transport	Salminen and Novick
	Heterotrimeric G-proteins implicated in traffic control	Melançon *et al.*
	Reconstitution *in vitro* of ER-to-Golgi transport	Becker and Balch
1988	Isolation of ER–Golgi intermediate compartment (ERGIC)	Schweizer *et al.*
1990	Application of brefeldin A to study Golgi–ER transport	Lippincott-Schwartz *et al.*
	Phosphoinositide 3-kinase implicated in control of Golgi traffic	Herman and Emr; Schu *et al.*
1991	Discovery of COPI coat	Duden *et al.*; Serafini *et al.*; Waters *et al.*
	Demonstration of role of Gαi3 in traffic control	Stow *et al.*
1993–1994	Demonstration that ER-to-Golgi transport is selective	Balch *et al.*; Mizuno and Singer; Rexach *et al.*
1994	Discovery of COPII coated vesicles	Barlow *et al.*
	COPI functions in Golgi-to-ER retrograde transport	Letourneur *et al.*

precise mechanism involved is still the subject of yet another Golgi controversy. In 1993, two models were introduced that now dominate the field: the kin-recognition model and the bilayer-mediated sorting model (reviewed in Ref. 45). The kin-recognition model holds that resident proteins of a particular cisterna interact to form large hetero-oligomers that prevent resident proteins from entering transport vesicles. The bilayer-mediated sorting model proposes that the length of the transmembrane domain is the crucial factor in sorting resident Golgi proteins, which have shorter transmembrane domains than those of the plasma membrane. It is proposed that Golgi membrane proteins are retained because they are excluded from cholesterol-rich regions of Golgi membranes destined for the plasma membrane. At present, the only aspect that appears to be agreed upon is the importance of the transmembrane domain in retention of Golgi-resident proteins.

The other key finding was that of Pelham[46] and colleagues, who demonstrated that receptor-mediated retrieval mechanisms are used for retention of resident ER proteins. They showed that, if resident proteins of the ER lumen escape and move down the secretory pathway, they are retrieved by a specialized C-terminal KDEL (or closely related) signal that is recognized by KDEL receptors located in the *cis* Golgi or ERGIC and transported back to the ER. Some ER membrane

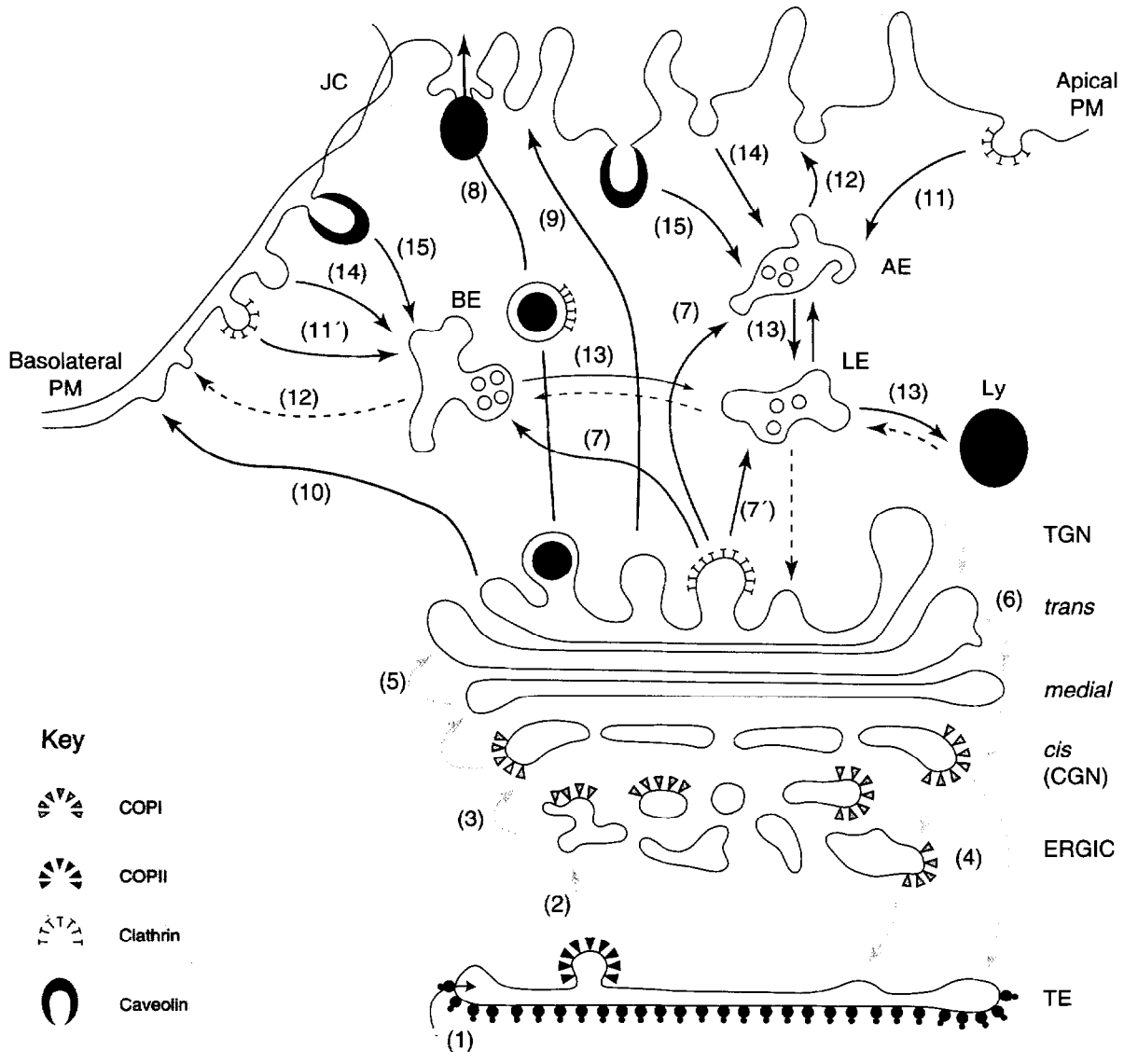

FIGURE 3

Diagram depicting the major routes of vesicular traffic to and through the Golgi apparatus along the exocytic (1–6) and endocytic (7–12) pathways. Exocytic pathways: secretory proteins, membrane glycoproteins and lysosomal enzymes are synthesized on polyribosomes and translocated to the endoplasmic reticulum [ER; (1)] where they undergo cotranslational and posttranslocational processing. They exit the ER via COPII vesicles (2), which serve to shuttle them to the ER–Golgi intermediate compartment (ERGIC, or vesicular–tubular clusters, VTCs). From there, they could be transported to the *cis*-Golgi network (CGN) via COPI-coated vesicles (3), but this is still controversial. COPI-coated vesicles also function in retrograde transport (Golgi to ER) (4). Subsequently, either the proteins traverse the Golgi cisternae one by one via vesicular carriers (5) or transport occurs by cisternal maturation. Retrograde transport is also assumed to take place between the stacked cisternae (6). Sorting occurs in the *trans* cisterna or *trans*-Golgi network (TGN). Lysosomal enzymes bind to mannose 6-phosphate receptors in the Golgi, are packaged into clathrin-coated vesicles in the TGN, and delivered either to early (7) or late (7′) endosomes. Membrane and secretory proteins are also sorted in the TGN and delivered by exocytosis along the regulated secretory pathway via secretion granules (8) or along the constitutive pathway (9). In polarized secretory cells, a separate pathway exists for delivery of vesicles to the basolateral domain (10). Endocytic pathways: the best-characterized endocytic pathway is receptor-mediated endocytosis through clathrin-coated vesicles budding from either the apical domain (11) or the basolateral domain (11′). Many receptors (low-density lipoprotein, transferrin) recycle back to the plasma membrane (PM) from early endosomes (12), whereas many ligands are transported from early to late endosomes to reach lysosomes (13). Other pathways include uptake in nonclathrin-coated vesicles (14) or by caveolae (15). Mannose 6-phosphate receptors shuttle between the Golgi and endosomes. Abbreviation: TE, transitional ER element. (Adapted, with permission, from Ref. 50.)

proteins possess either a KKXX (type I) or XXRR (type II) retrieval signal that binds to COPI (Ref. 41). Also, a tyrosine-based sorting signal is used for retrieval of a TGN protein, TGN38, from the plasma membrane. Thus, the existence of both retention and retrieval signals for resident ER and Golgi proteins has been validated.

Role of the Golgi in sorting and packaging

A role for the Golgi apparatus in the formation of secretion granules was suggested as long ago as the 1920s based on observations on glandular cells by light microscopy. Direct evidence for the role of the Golgi in concentration and packaging of secretory proteins was provided by the work of Palade's

laboratory on the exocrine pancreas[4] and was substantiated subsequently by work on many other cell types during the golden era of electron microscopy in the 1960s and 1970s. At about the same time, it was recognized, on the basis of acid phosphatase enzyme cytochemistry, that lysosomal enzymes are transported to endosomes in coated vesicles that bud from the *trans* or exit side of the Golgi[14]. An important milestone in understanding Golgi sorting functions was the recognition in 1985 by Moore and Kelly[47] of the existence of two distinct secretory pathways, regulated and constitutive, and the demonstration that secretory proteins are selectively sorted from membrane proteins, with the former packaged into dense-cored granules and the latter into constitutive secretory vesicles in the *trans* Golgi. Thus, it became evident that sorting of Golgi cargo for distribution to other cellular sites occurred in the last cisterna of the stack, which had a distinct morphology and was given several names including GERL, *trans* Golgi reticulum and *trans*-Golgi network or TGN (Ref. 16). Among these, only TGN has survived.

Later on, it was recognized that there are two types of constitutive secretory vesicles destined for different plasmalemmal domains that bud from the TGN. Originally identified in polarized epithelial cells[48,49], they were more recently found also to exist in nonpolarized cells. Thus, it became apparent that newly synthesized proteins are sorted in the TGN and selectively packaged into different containers. Four such containers have so far been recognized and well characterized: secretion granules, two types of constitutive secretory vesicles, and clathrin-coated vesicles carrying lysosomal enzymes. Several other putative vesicle populations (e.g. p200 vesicles and AP-3 vesicles) have been described, but exactly what they are doing remains controversial (reviewed in Ref. 50).

The first sorting signal: mannose 6-phosphate

A landmark discovery familiar to most cell biologists was the discovery of the mechanism of sorting of lysosomal enzymes through the mannose 6-phosphate (M6P) sorting signal. This came about as the result of work in the late 1970s from several laboratories, including those of Kaplan and Sly, and Kornfeld and Jourdian (see Ref. 51 for a review), demonstrating that lysosomal enzymes possess phosphorylated mannose residues recognized by M6P receptors in the TGN, leading to their selective removal from the exocytic pathway. This discovery demonstrated that sorting inside the cell occurs by a process resembling receptor-mediated endocytosis at the plasma membrane and became the paradigm that has guided thinking about mechanisms of intracellular sorting to this day. Surprisingly, although more than 20 years have passed since the discovery of the first intracellular sorting signal, the mechanisms of sorting at the TGN for biosynthetic products other than lysosomal enzymes are still poorly understood. Sorting into regulated granules is believed to occur by aggregation and sorting of at least some proteins into constitutive vesicles by tyrosine-based signals. In addition, a unique mechanism was suggested recently by Simons and Ikonen[52] for sorting membrane proteins destined for the apical domain of the plasma membrane involving lipid rafts and a galactose-binding protein, VIP36.

A continuing controversy: does transport across the Golgi stack occur by cisternal maturation or vesicular transport?

Since the 1960s and 1970s, it has been clear that cargo moves across the Golgi stack, but the mechanism by which this takes place has been debated for over 40 years. Two models have been proposed. The first was the maturation or cisternal progression model introduced in the 1950s[53], which visualizes the Golgi as a kind of bottling station: cisternae are formed on the entry or *cis* face and move sequentially towards the exit or *trans* face, where they are used up in packaging. This concept has been championed by Morré[54] and Leblond[55] among others.

New information obtained during the 1970s, especially the demonstration of the distinctive composition of Golgi compartments, the faster turnover of secretory versus membrane proteins and the existence of recycling, was difficult to reconcile with the maturation model. The vesicular transport/stationary cisterna model, introduced in 1981 (Ref. 11), was designed to take into account this new information. It held that each cisterna (or set of cisternae) constitutes a separate compartment of distinctive composition and that transport from one cisterna to another occurs through vesicles. The vesicular transport/stationary cisterna model gained wide support based on results obtained from *in vitro* systems, and was championed by Rothman and coworkers, whose *in vitro* experiments were interpreted as reconstituting anterograde vesicular transport[40,56,57]. As a result, the vesicular transport model has dominated the field for over 15 years. Recently, however, it has been seriously challenged[58] as the result of several developments, including the failure to identify t-SNAREs (see below) associated with intra-Golgi transport, the failure[59] until recently[60] to detect secretory proteins in Golgi vesicles, and the difficulties in applying the vesicular transport model to the maturation of complex structures such as algal scales[61], procollagen fibrils[55] and casein[62] that are found across the Golgi stack.

Once again, new data and resurrection of some old data have stimulated a re-examination of existing models. As a result, the maturation model in modified form, which is actually a hybrid between the maturation and vesicular transport models, is enjoying a strong comeback. It holds that anterograde transport occurs by cisternal maturation coupled with retrograde vesicular transport of Golgi enzymes. There are many aspects of this modified cisternal progression model that appear attractive, but its key features – anterograde transport of individual cisternae, retrograde transport of Golgi enzymes, and *de novo* formation of *cis* Golgi cisternae – remain to be demonstrated convincingly. Similarly, a key feature of the stationary cisternae model – the existence of anterograde vesicular transport – has been seriously challenged[58]. Therefore, it is safe to say that neither the vesicular transport model nor the modified maturation model for anterograde transport is

completely proven and that the mechanism of transport across the stack remains controversial. As long as the evidence is conflicting or not entirely convincing, this controversy will continue. The lessons learned from Golgi history suggest that it will take new approaches and convergent information from different quarters to design universally accepted, accurate models.

Recyling, retrograde transport and tubules

The likely existence of recycling of membranes involved in transport to and through the Golgi was predicted at the time of the discovery of vesicular trafficking[4]. The first convincing evidence for reutilization or recycling of Golgi-derived membranes was obtained using particulate, electron-dense tracers (dextrans and cationized ferritin) to mark recovery of membranes of secretion granules, their recycling to the *trans* Golgi, and reutilization in packaging of secretory proteins[63,64]. It may seem hard to believe, but this, too, was the subject of controversy because at that time it was assumed, based on the studies with the fluid-phase marker horseradish peroxidase, that plasma membrane proteins recycle through endosomes and that there was no access to the Golgi from the plasma membrane. Doubts lingered until the demonstration[65] that, when plasma membrane proteins are desialylated at the cell surface, they can be resialylated during recycling through the Golgi. In the meantime, it became evident that, as the half-life of most membrane proteins is rather long (1–3 days), recycling must occur at all steps in intracellular transport[11] and that transport between the Golgi and other compartments to which its cargo is delivered (lysosomes, plasma membrane, endosomes) involves both anterograde and retrograde pathways (see Fig. 3).

Until recently, however, most studies focused on anterograde transport, but studies with brefeldin A (BFA) focused attention on retrograde transport and brought to light the potential role of tubules as well as vesicles in transport from the Golgi to the ER[66]. When cells were treated with BFA, tubules formed through which Golgi components relocated to the ER. Tubules were also seen as components of the ERGIC and VTCs in normal cells. Recent work in which cargo is tagged with green fluorescent protein (GFP) suggests a wider involvement of tubules in anterograde transport both between the ER and Golgi and between the Golgi and the plasma membrane than had been appreciated until now[67].

Mechanisms of vesicle budding, targeting and fusion

Since the realization that proteins could be transported to and through the Golgi by vesicular transport, investigators have been intrigued to know how vesicles bud and recognize and reach their target. Progress in this arena was slow and was not linked to a single discovery or event. Significant insights have been obtained only in the past four years as a result of the convergence of information derived from three seemingly disparate sources: biochemical studies on Golgi transport *in vitro*[40,57], analysis of yeast *sec* mutants[42,68–70] and characterization of synaptic vesicle proteins[71,72]. It became evident that vesicles involved in transport at different steps along the exocytic pathway have common features in terms of their fusion machinery but apparently specific targeting equipment. Most have prominent coats of which there are at least three types – COPII, COPI and clathrin (Fig. 3) – plus several less-well-characterized new candidates, including AP-3 and p200[73,74]. COPI and COPII vesicles are involved in transport between the ER and Golgi, and clathrin-coated vesicles are of two types: those involved in transport of lysosomal enzymes between the TGN and endosomes and those involved in receptor-mediated endocytosis at the plasma membrane. The model for formation of all these vesicles was provided by clathrin-coated vesicles[73,74]. Based on this model, vesicle budding is thought to be initiated by the assembly of the protein coat, triggering assembly of elaborate membrane and cytosolic protein complexes, unique for each vesicle population, that drive vesicle budding and fission. Once formed, cargo proteins, cargo receptors and membrane proteins required for target recognition are included in the vesicle.

How do vesicles recognize and fuse with their appropriate target? The prevailing working model is the SNARE hypothesis introduced in 1993 (Ref. 75) when it was realized that synaptic vesicles and intra-Golgi transport vesicles have similar components. According to this model, pairs of integral vesicle and target membrane proteins (v- and t-SNAREs), with specific family members assigned to different stations, ensure docking to the appropriate membrane receptor[40,57]. Soluble cytosolic proteins – *N*-ethylmaleimide-sensitive fusion protein (NSF) and soluble NSF attachment proteins (SNAPs) – serve as common fusion machinery for different vesicle relays. Rab proteins are believed to check the fidelity of the membrane-fusion and -targeting event. Recognition by specific v- and t-SNAREs is assumed to apply to most but not all vesicular transport steps along both the exocytic and the endocytic pathways. An exception is represented by the vesicles involved in apical delivery of Golgi-derived proteins in polarized secretory cells where glycolipids and glycans appear to be involved[52].

Several controversies currently pervade this area of Golgi research. First, it is debated whether COPI vesicles are involved in both anterograde and retrograde transport between the ER and Golgi or only in retrograde transport. Second, it is questioned whether COPI vesicles are involved at all in anterograde intra-Golgi transport. Indeed, as indicated earlier, the very concept of anterograde vesicular transport within the Golgi is currently being contested[58]. Third, the SNARE hypothesis has recently been attacked by some as being too simplistic, which is probably the case. One has only to remember that synaptic vesicles contain dozens of proteins[72] and the function of relatively few of these has been established. Moreover, from the work in yeast, we begin to have an inventory of many of the genes involved in sorting and in vesicle formation and targeting. In the case of COPII vesicles, for example, at least nine proteins besides SNAREs are proposed to be involved in sorting, coat assembly and vesicle budding[42].

How are Golgi functions and Golgi traffic regulated?

This question has only begun to be tackled in the past 5–10 years and was made possible by availability of *in vitro* transport assays in both mammalian cells and yeast and by analysis of yeast mutants. Evidence is accumulating that small GTPases of the Ras superfamily, heterotrimeric G proteins, and phosphoinositides, are involved. Most is known about the small GTPases. Their involvement in vesicular traffic was first indicated in 1987 when Salminen and Novick[76] discovered a small Rab GTPase, Sec4p, which is required for transport from the Golgi to the cell surface in yeast. This finding was extended very rapidly, and subsequently a large (20–30) family of small GTPases, the Rabs, was discovered, each with a characteristic distribution in the cell[70,77,78]. Of these, Rab1A and Rab2 are located on ERGIC (VTCs) and the *cis* Golgi, and Rab6 is located in the *trans* Golgi. Rab1A and another small GTPase, Sar1p, were shown to be essential for ER-to-Golgi transport. Rabs are believed to carry out a proofreading function, checking that each vesicle fuses with the appropriate target[70,77]. Originally it was believed that each Rab was dedicated to a specific step in transport, but now there are more Rabs than defined steps.

Heterotrimeric G proteins were first implicated in control of vesicular trafficking by the finding of Melançon *et al.*[79] that AlF^-, a specific inhibitor of heterotrimeric G proteins, inhibited ER-to-Golgi transport *in vitro*. Indirect evidence suggested the involvement of trimeric G proteins in virtually every transport step[80,81]. Subsequently, several G proteins, notably Gαi3[82–84] and Gαs and Gαq[84], were localized to Golgi membranes. The most direct findings documenting involvement of G proteins in Golgi traffic control were those of Stow *et al.*[82], who showed that overexpression of Gαi3 inhibited processing of a secretory protein, heparan sulfate proteoglycan, presumably by inhibition of transport through the Golgi. The precise role of G proteins – whether in coat assembly, sorting, vesicle budding or vesicle fusion – remains unknown. Trimeric G proteins are well known to regulate signalling at the plasma membrane by interaction with various effectors. The presence of trimeric G proteins on intracellular membranes, especially those of the Golgi apparatus, suggests that they could have similar functions inside the cell. However, it is not clear whether the effects occur via classical or nonclassical G-protein pathways.

The first indication of a role for phosphoinositides in vesicular trafficking came from the discovery by Emr and coworkers[85,86] of the yeast *VPS34* gene, whose product is required for transport of vacuolar proteins from the Golgi to the vacuole, which is a lysosome equivalent. Vps34p was shown to possess extensive homology with bovine phosphoinositide 3-kinase. Indirect evidence was obtained subsequently, by use of the inhibitor wortmannin, for the involvement of a Vps34p-like protein in transport from the TGN to the Golgi in mammalian cells[87,88]. The mechanism by which phosphoinositides control vesicular traffic has not been established, but recent evidence suggests that they could regulate the activity of proteins with diverse functions such as dynamin, AP-2 and guanine-nucleotide exchange factors[89]. In short, delineation of mechanisms for control of vesicular traffic is in its infancy and provides problems of sufficient magnitude to keep an army of investigators busy for perhaps another 100 years.

Future perspectives

The first century of the history of the Golgi complex has been one of great progress, yet it has also been fraught with controversy. It was not until 1954 that the Golgi itself was accepted as a bona fide organelle. Since that time, controversy has surrounded the Golgi, and it continues to this day. What lessons can be learned from past history? First, it is clear that problems are not resolved by heated discussions, introduction of premature dogmas, intimidation or suppression of alternative points of view. Second, from the Golgi milestones reviewed here, it is evident that controversies that have arisen were often resolved by information that came about as a result of new technical developments such as the introduction of the electron microscope in the 1950s, of enzyme cytochemistry, autoradiography and cell fractionation in the 1960s and 1970s, and of recombinant DNA technology, *in vitro* assays and yeast mutants in the 1980s and 1990s. Third, only when the evidence is strong, and complementary approaches converge and lead to the same conclusions, can models be formulated that stand the test of time.

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Acknowledgements

The authors are supported by NIH grants CA58689 and DK17780 (to M. G. F.).

Golgi centenary meeting

To celebrate the Golgi centenary, there will be a special meeting later this year in Pavia, Italy, the place where Golgi carried out the majority of his investigations.

Structure and function of the Golgi complex: state of the art 100 years after Camillo Golgi's discovery
19–23 September 1998
University of Pavia, Italy
Organizers: Kathryn Howell, Alberto Luini, Antonieta de Matteis and Alexander Minorov

The invited speakers will include:
W. Balch, V. Bankaitis, J. Bergeron, J. Bonifacino, P. de Camilli, S. Emr, M. Farquhar, B. Glick, H-P. Hauri, W. Hong, K. Howell, T. Kreis, J. Lippincott-Schwartz, V. Malhotra, P. Melançon, J. Meldolesi, D. Morré, J. Morrow, S. Munro, P. Novick, G. Palade, H. Pelham, A. Rambourg, J. Rothman, R. Schekman, K. Simons, A. Staehelin, G. van Meer, G. Warren, M. G. Waters, F. Wieland and M. Zerial.

For information contact:
Ms Anna Cavallo
Secretariat, Golgi centenary meeting
Consorzio Mario Negri Sud, Via Nazionale, 66030 S. Maria Imbaro (Chieti), Italy
Tel: +39 872 570 338 • Fax: +39 872 578 240 • E-mail: cavallo@cmns.mnegri.it