The Golgi complex: perspectives and prospectives

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Abstract

We now have considerable understanding of the role of the Golgi complex in the posttranslational modifications of membrane and secretory proteins and of lysosomal hydrolases. It is now also clear that the Golgi plays a key role in the intracellular packaging, addressing, and sorting of these classes of proteins to their final destinations on the secretory and endocytic pathways. While it has been proposed that vesicular budding and fusion underlie entry of proteins into the Golgi from the ER and subsequent movement among its cisternae and exit to their final stations, recent observations indicate that this model may need to be revised based on studies in living cells where vesicular-tubular structures appear to mediate membrane trafficking. This will be a major challenge for investigators in the coming years who will rely again on the use of morphologic techniques of the sort that started it all in 1898. ß 1998 Elsevier Science B.V. All rights reserved.

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Camillo Golgi first saw his namesake in 1898 in heavy metal impregnated brain tissue but it was only many years later that the true impact of his observation became clear. The Golgi complex – named for the heterogeneous collection of vesicles and cisternae that early electron microscopists observed – went through many iterations: it was initially regarded as an artifact of osmium fixation then later a curiosity among cell organelles as electron microscopists began to identify and catalogue structures in diverse cell types [1,2].

A major advance in understanding of the Golgi complex came in 1961 when Novikoff and coworkers [3] and many others utilized electron microscopic cytochemistry which clearly showed that the cisternae were heterogeneous in their composition suggesting that specialization of function existed across the stacks. At about the same time, light and electron microscopic autoradiography were being applied to intact organisms and tissues. One landmark finding was that the Golgi complex was extremely active in the incorporation of sugars [4] which eventually led to the identification of one of the exclusive functions of this organelle: processing of the terminal sugars in N-linked glycoproteins and the synthesis of O-linked oligosaccharides including terminal sulfation of glycoproteins and proteoglycans. Subsequent development of electron microscopic autoradiography identified, in major exportable protein synthesizing cells such as the pancreatic acinar cell, an essential role for the Golgi in the intracellular transport of secretory proteins and their concentration and storage in secretory granules prior to release by regulated exocytosis [5,6]. (It was not until several years later that the Golgi was also found to play a similar role in the maturation of membrane proteins.) While these were important observations in determining the function...
of the Golgi complex, it was also believed even at that time that non-glycoprotein secretory proteins bypassed the Golgi and exited the cell directly from the rough endoplasmic reticulum or even exited the cell by crossing into the cytoplasm from the RER and crossing membranes directly.

It is important to also note here that the biochemists were not inactive during this period. Early attempts to isolate the Golgi complex by cell fractionation were tedious because of the absence of reliable biochemical markers. Success of fractionation had to rely on electron microscopy of the fractions, but in 1969–1970 several groups reported that glycosyltransferase activities were concentrated in Golgi fractions which made realistic the possibility of isolation of Golgi subfractions allowing investigators to further define the multiple functions of the Golgi [7–9].

At that time (1960s–1970s), a number of conclusions could be made concerning the function of the Golgi complex. (1) It appeared to be obligatorily involved in the intracellular transport and processing of secretory proteins, both glycoproteins, and non-glycosylated proteins and peptides. (2) Soluble proteins synthesized in the RER remained in the confines of membrane-bounded compartments until secreted by exocytosis and never possessed a soluble cytoplasmic phase after encapsulation in the RER lumen and transfer to the lumina of Golgi cisternae. (3) As a consequence of 1 and 2, it was necessary to propose mechanisms for the transfer of proteins from the RER to the cisternae of the Golgi complex. Transport over this segment of the secretory pathway was postulated to take place by participation of the ‘clouds’ of smooth surfaced vesicles located between the RER and the so-called entry face of the Golgi. It was also proposed that proteins gained access to these transport vesicles by pinching off of ‘transition al elements’ of the RER – smooth surfaced protrusions of the RER that faced the Golgi entry side and contained a dollop of ER content and also a portion of the RER membrane. Conjecture as to how the composition of the RER and Golgi membranes were maintained included selective retrograde retrieval or recycling [5,6,10]. This model of RER-Golgi communication was in agreement with the results of cell fractionation in which a smooth vesicular fraction with kinetic properties consistent with a role in ER to Golgi communication could be isolated. It was also compatible with electron microscopic images showing smooth surfaced vesicles strategically located between the RER and the Golgi complex [5]. However, it should be pointed out here that this model of intracellular transport did not rule out continuous, tubular interconnections and it was well known at the time that mechanical damage of cells such as occurs during cell fractionation or which takes place because of osmium fixation leads to vesiculation of the RER and other membranes. Some contemporary electron microscopists were champions of the tubular interconnection versus vesicular concepts of organization of the para-Golgi membranes. Novikoff and colleagues proposed tubular connections from the RER that bypassed the Golgi which were involved in transport of lysosomal hydrolases (GERL, Golgi-ER-lysosomes) while the methodical serial reconstruction of thin section images by Rambourg and Clermont [11] indicated that both the RER to Golgi region and the cisternae of the Golgi proper possessed extensive continuous, anastomosing interconnections.

At about the same time (1970s–1980s), electron microscopic histochemical and immunocytochemical techniques were refined and revealed a rich diversity of enzymatic functions among the cisternae of the Golgi complex, leading to a vastly expanded view of the functions of the Golgi in processing of proteins. Especially significant was the finding that the cisternae of the Golgi were ‘polarized’ in organization from the cis (formerly called the entry or forming side) to trans (exit or maturing) (see [2,12]). The observation that secretory proteins are concentrated and stored in secretory granules in the vicinity of the trans-Golgi pointed out the potential role for the this organelle in sorting proteins into the regulated exocytic pathway. Finally, in 1978, the elegant studies of Sabatini and coworkers (among others) [13] clearly showed that membrane proteins also pass through and are processed by the Golgi. These studies also cemented the idea that an additional major function of the Golgi was to serve as a sorting station for plasma membrane proteins that end up in the apical or basolateral domains of polarized epithelial cells. These investigators had used the coat glycoproteins of enveloped viruses that bud either apically or basolaterally from polarized cells as markers for membrane glycoprotein sorting. Shortly thereafter, it was
found that lysosomal hydrolases are sorted from the Golgi to lysosome by means of a specific receptor mediated process (summarized in [14]).

By 1981, the Golgi complex had moved from the level of artifact and curiosity to 'center stage' which is thoroughly and thoughtfully chronicled in a review by Farquhar and Palade that includes this phrase in its title [1]. Rapid advances in cell fractionation, autoradiography and immuno- and histochemistry had produced a plethora of observations which posed many questions of a mechanistic nature. Many of the chapters in this volume address these questions. A partial list follows. This list is evidently incomplete and reflects the author's views (and biases).

1) Intracellular transport. Earlier studies, summarized above, provided some of the basis for the hypothesis that transport from the RER to the Golgi complex and subsequent communication between its cisternae utilized discrete vesicular transport steps. This model assumes that the cisternae remain stable and communication between their lumina is effected by vesicular carriers. (The membrane flow [15] or cisternal conversion model is now less generally accepted in view of the observations of discrete biochemical domains among Golgi cisternae.) The now classic studies of Rothman and colleagues [16] put this model to direct test in vitro and strongly suggested that vesicular intermediates account for intra Golgi communication. This was a conceptually powerful hypothesis that has driven many subsequent studies on interactions among membrane bounded compartments. However, it is clear that this hypothesis may need to be modified on the basis of recent observations on RER and Golgi membrane dynamics in living cells indicating the involvement of tubular interconnections. This is discussed below along with caveats that may reconcile the two sets of observations that superficially appear to be at odds.

More recent studies have further refined and redefined the cis and trans domains of the Golgi, both morphologically and functionally. Evidence that the cis and trans sides of the Golgi may consist of tubular interconnections is clearly consistent with recent observations on living cells (these areas were termed the CGN and TGN, respectively, because of their morphologic appearance under certain conditions). It should be pointed out that these new terms are in part redefinitions and embellishments of Novikoff's GERL. They also are a vindication of the work of Rambourg and Clermont whose serial section reconstruction of the Golgi complex are still among the most informative [11]. Questions that arise anew pertain to the extent that fixation has modified morphology both by causing vesiculation or by generating tubular interconnections.

Finally, it has become clear that the Golgi complex is indeed at the center of the cell’s stage in that it not only mediates the outward pathway of secretory and membrane proteins, but also interacts dynamically with the incoming endocytic limb [17]. What determines how endosomes interact with the Golgi and to what depth in the secretory pathway these interactions extend remains to be determined.

2) Recycling. The observations that the membranes of the Golgi possessed chemical and enzymatic properties quite distinct from the donor compartment, the RER, and from the recipient compartments, secretory granules or lysosomes/endosomes, raised the question as to how membrane composition of these three compartments could be kept distinct in the face of extensive dynamic interactions during vesicular transport of cargo and given the rapid diffusion of membrane proteins and lipids in the plane of the membrane. As discussed in this volume, it is now evident that the unique properties of both the donor RER and the recipient cis-Golgi is accounted for by selective retrieval or recycling of ER membrane from the Golgi. Elegant studies using yeast have defined this mechanism and the same recycling device appear to be employed by mammalian cells. A similar situation must occur amongst the cisternae of the Golgi in order to preserve their unique membrane compositions. Likewise, exocytosed secretory granule membrane must be retrieved from the cell surface, possibly to the Golgi complex in order to preserve the composition and area of the plasma membrane. We must remember, however, that the physical basis for selective membrane retrieval may need to be modified in the future in view of the observations (see later) of extensive tubular interconnections along the RER-Golgi-secetory vesicle pathway.

3) Sorting from the Golgi. Earlier studies on the pathway that secretory and lysosomal proteins take
through cells and the collective observations that secretory and membrane proteins destined for terminals along the secretory pathway are intermixed in the trans-Golgi, raised the important concept that the TGN must somehow be involved in the sorting and delivery of proteins along post Golgi routes [18]. Sorting of lysosomal hydrolases from the TGN to lysosomes using a receptor mediated process [14,17] was the impetus for searches for similar receptor-mediated sorting for extracellular secretory proteins and membrane proteins from the TGN. So far, no further convincing examples of receptor-mediated sorting have been uncovered although now it is clear that in several systems, specific aggregation or multimerization of proteins precedes their entry (sorting) into the regulated secretory pathway, an event that may be based on physical properties per se. By exclusion, proteins that enter the constitutive secretory pathway are believed to be unsorted or subject to bulk flow though this concept should not necessarily imply lack of processing or absence of regulation. The mechanisms that distribute membrane proteins from the TGN to compartments along the endocytic and exocytic pathways and to apical and basolateral plasmalemmal domains await clarification. It is becoming evident, however, that large scale sorting of some membrane proteins from the TGN may entail distribution into rafts of membrane lipids that in turn carry their protein passengers to the correct domain [19,20]. The precise role of the Golgi in lipid metabolism and the role of lipids in sorting deserves further investigation.

(4) Mechanisms of vesicular transport. Early on in studies on the Golgi complex, it was observed that certain regions of the TGN, especially those adjacent to forming secretory granules, possessed a clathrin coat which was suggested to be involved in intracellular transport. Morphologic studies on several systems also showed other types of membrane ‘coats’ on the cytoplasmic side of vesicles in the cis side of the Golgi complex [21]. Subsequent studies, using selective Golgi disrupting agents such as brefeldin [22], have now clearly determined that many of these ‘coats’ serve to induce the curvature that is required for the pinching off of vesicles. Again, how membrane coats fit into a tubular vesicular model of intracellular transport is a challenge for the future. One of the major concepts to arise from studies on the Golgi complex and other steps in the exo-endocytic pathways is the so-called SNARE hypothesis. Simply put (the topic has been extensively reviewed recently [23]), each interacting membrane pair possesses a specific donor vesicle membrane protein (t-SNARE) that recognizes the recipient membrane domain which carries a specific recognition protein [24–26]. Complementary interactions between v- and t-SNAREs determine specificity of interactions among vesicles. This model has been convincingly verified for synaptic vesicle exocytosis [27]. The role of the SNARE hypothesis in transport through the Golgi complex is becoming clear as expected from the conserved generality of the concept [26]. However, we must again reconcile this hypothesis, which in its initial iteration pertained to discrete vesicular interactions, with a modified tubular vesicular mechanism for intracellular transport (see Discussion in [28]).

Which brings us to the present. A provocative recent study by Lippincott-Schwartz and colleagues [29] has examined, in live cells, the intracellular transport of VSV-G protein tagged with green fluorescent protein. When activated by UV light in situ, it is possible, using high resolution confocal microscopy, to observe transport from the RER to the Golgi and onward toward the cell surface. What this remarkable study shows is that interconnections between the RER and Golgi occurs from multiple random sites on the RER and significantly, many of these interconnections are through ‘vesiculo-tubular clusters’ [29]. The work also shows clearly that all of the cisternae of the Golgi complex appear to be permanently interconnected from a series of photo bleaching recovery images. This study, along with others that preceded it in cultured CNS cells whose Golgi lipids had been tagged with exogenously administered fluorescent ceramide [30], has suggested that vesicular transport mechanisms may not be the norm (discussed in [28]). They are also interpreted as ruling out vesicular budding of transitional elements from RER to Golgi transport vesicles. Not to downplay the importance of these observations, it needs to be kept in mind that the resolution of light microscopy may not allow visualization of small vesicles and so their role in transport cannot at present be ruled out. Both mechanisms may therefore be operative. It will also be important to repeat these important studies in polarized epithelial cells rather than
the extremely flat CHO cells where pathway lengths between the Golgi and the plasma membrane are very short and in which organization of the RER relative to the Golgi may differ from that in epithelial cells. Nonetheless, this type of study on intact, live cells serves to reunite older observations on cells examined by classic morphologic techniques. Significantly, they serve to introduce new questions whose answers surely will come from the recognition of the conservation of the function of the Golgi complex over evolutionary time and application of the power of molecular genetics to problems as ‘complex’ as the Golgi can throw at us. Maybe we should subtitle this collection of essays ‘The Golgi Complex; from Center Stage to Full Monty’.

References
