The organisation of the Golgi apparatus

Graham Warren* and Vivek Malhotra†

The past year has seen considerable progress in understanding the mechanism of COPI (coatomer protein I) vesicle docking and SNARE (soluble NSF attachment protein receptor) mediated fusion, the mechanism of cisternal growth and stacking and the regulation of Golgi architecture. The route taken by cargo proteins through the Golgi apparatus is still a matter of some dispute.

Addresses
*Imperial Cancer Research Fund, PO Box 123, 44 Lincoln's Inn Fields, London WC2A 3PX, UK; e-mail: g.warren@europa.lif.icnet.uk
†Department of Biology, University of California at San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA; e-mail: malhotra@jeeves.ucsd.edu

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Abbreviations

COPI	coatomer protein I
ER	endoplasmic reticulum
ERK	extracellular signal regulated kinase
GRASP65	Golgi reassembly stacking protein of 65 kDa
IQ	ilimaquinone
MAPK	mitogen-activated protein kinase
NSF	N-ethylmaleimide sensitive factor
SNAP-25	synaptosomal associated protein of 25 kDa
SNAP	soluble NSF attachment protein
SNAREs	SNAP receptors
TGN	*trans*-Golgi network

Introduction

This is the centennial year of the discovery of the Golgi apparatus, one of the first intracellular organelles to be visualised. It was observed by several workers at the end of the last century, but Camillo Golgi published first [1]. The unique architecture of this organelle was not revealed until the advent of electron microscopy in the early 1950s [2•] yet the underlying basis of this architecture, its regulation and even function are only just beginning to be appreciated. In this review, we will focus on the transport of cargo through the Golgi apparatus at the molecular level, where progress continues to be swift, and at the gross level, where progress is slow and there is still no agreement even as to the route taken by cargo through the Golgi stack. We will also focus on the structure of the Golgi apparatus and the progress made in identifying proteins that regulate and determine its unique architecture.

Vesicle flow patterns

The controversy as to how cargo proteins move through the Golgi stack continues to dominate the minds if not the experiments of workers in the field. There are now more models and speculations than primary literature and it sometimes seems as if all conceivable modes of getting through the Golgi apparatus have been put forward at one time or another [3,4•,5,6,7•,8•,9]. Although it is not our intention to add to the speculation, we thought it worth summarising most of the arguments put forward for the two most popular models: cisternal maturation and anterograde vesicle transport (Table 1; Figure 1). The former argues that the cargo stays put and Golgi enzymes are delivered at the appropriate time and in the appropriate order by retrograde transport so that each cisterna 'matures' into the next. The latter argues that it is the Golgi enzymes that stay put in their appropriate and different cisternae and the cargo is delivered to each cisterna in turn by anterograde vesicle transport.

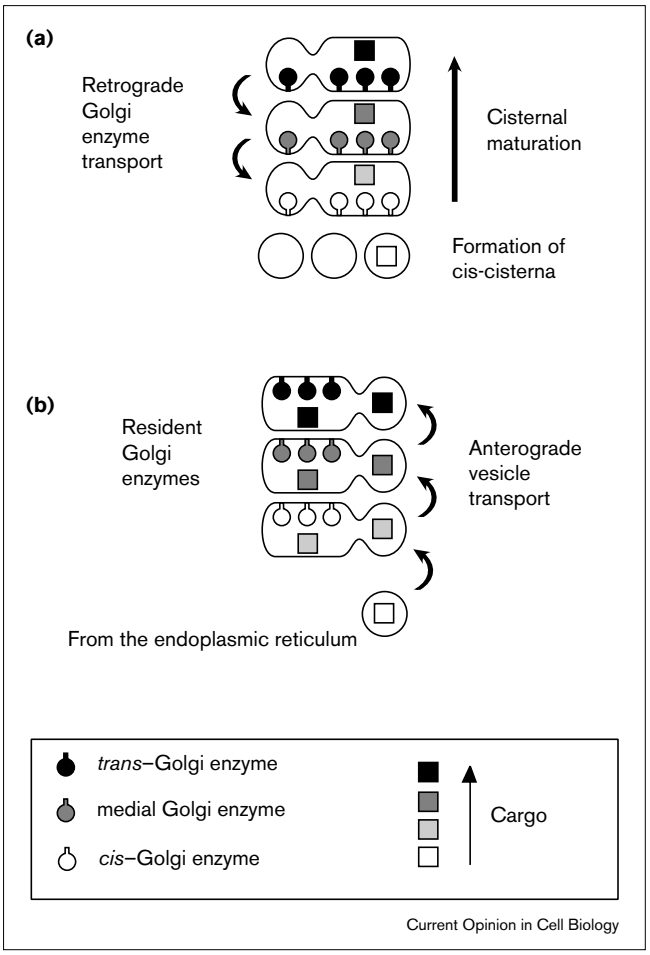
Though our personal bias favours anterograde vesicle transport, we have to conclude that none of the available data are overwhelmingly convincing and a more definitive set of experiments is needed. The most obvious would be based on those observations that provided, and still provide, the cisternal maturation model with its strongest evidence. Algal scales are too large to be transported by

Table 1

Cisternal maturation versus anterograde vesicle transport.

Evidence for cisternal maturation	References
The Golgi apparatus in certain algae can transport scales too large for vesicular transport	[3]
Mutations in yeast coatomer subunits affect retrograde not anterograde transport	[54•,55]
Golgi transport vesicles contain resident Golgi enzymes	[56,57•]
There are too few SNAREs in the yeast genome to account for multiple anterograde transport steps through the Golgi apparatus	[4•]
cis-Golgi membranes may be formed by the fusion of pre-Golgi intermediates (vesicular-tubular clusters)	[58•,59•]
Evidence for anterograde vesicle transport	
The Golgi apparatus has a defined structure comprising a fixed number of cisternae bounded by *cis*- and *trans*-Golgi networks. Golgi enzymes have fixed and discrete locations	[60–63]
COPI vesicles carrying anterograde cargo will fuse with target Golgi membranes	[64]
Members of the p24 family of COP receptors have either anterograde or retrograde signals	[65•,66]
Two populations of COPI vesicles can be distinguished containing either anterograde (pro-insulin) or retrograde (KDEL receptor) cargo but not both	[67]

Figure 1



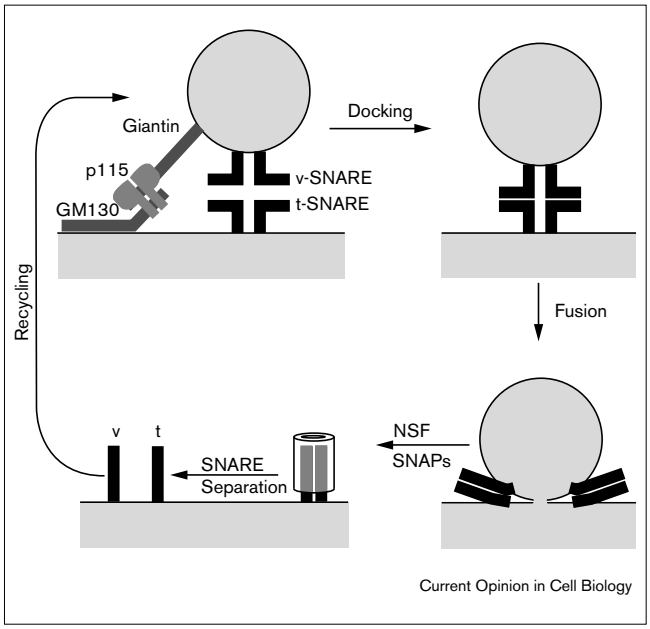
The two most popular models of intra-Golgi transport. **(a)** Cisternal maturation, in which each cisterna carrying resident cargo matures by the retrograde transport of Golgi enzymes. **(b)** Anterograde vesicle transport in which the Golgi enzymes are resident and the cargo moves forward in vesicles.

vesicles [3] but the counter-argument was that the earliest cisternae had no scales and these constituted the Golgi apparatus proper. The membranes containing the scales were simply post-Golgi transport vesicles that looked like cisternae because of the shape of the cargo they contained. If it were possible to introduce, into the *cis*-most cisterna, a structure too large to move by anterograde vesicles, this would provide definitive evidence: if the structure moves, cisternal maturation must be correct; if it doesn't, but smaller cargo moves around it, then anterograde vesicle transport must be the mechanism. The technical problem will be to introduce (or irreversibly assemble) a large structure in the *cis*-most Golgi membranes.

Vesicle transport

Though there is general acceptance of the SNARE (soluble NSF attachment protein receptor) hypothesis [10] and the role played by NSF (N-ethylmaleimide sensitive factor) in breaking up pairings of vesicle (v) and target (t) SNARES. [11], the timing of this event has been the subject of debate [12]. NSF was originally thought to bind to SNARE pairs via SNAPs (soluble NSF attachment proteins) bridging the vesicle and target membrane and ATP hydrolysis was then somehow linked to membrane fusion. Later work suggested that break-up of the SNARE pairs was not directly linked to membrane fusion. Instead, the SNARE pairs were broken up after the fusion event, thereby priming them for the next [12] (Figure 2).

Figure 2



Model for the docking and fusion of COPI vesicles. The tethering of COPI vesicles by proteins including GM130 (Golgi matrix protein of 130 kDa), p115 and giantin leads to the pairing of v- and t-SNAREs, which brings the membranes sufficiently close together for membrane fusion to occur. NSF then catalyses the unfolding of the SNARE pair and the v-SNARE is recycled for further rounds of docking and fusion.

Rothman and colleagues [13•] have now shown that the SNAREs alone constitute the minimal fusion machinery. They reconstituted v-SNAREs (VAMP/synaptobrevin) and t-SNAREs (syntaxin 1 and SNAP-25) into separate liposomes and showed that these would fuse with each other but not with themselves. SNAREs are coiled-coil proteins and v-SNAREs and t-SNAREs are known to assemble in a parallel orientation with their membrane anchors at the same end [14•]. This means that as a v-SNARE in a vesicle coils up with a t-SNARE in the target membrane, the two membranes will be brought close together (see Figure 2). Furthermore, the formation of one SNARE pair should encourage the formation of others that should spontaneously assemble a rosette surrounding the two membrane regions that are brought into contact with each other. Simply by bringing these two membranes close enough together to exclude water might

then suffice for fusion to occur. The SNARE pair, now in the same membrane, is an extremely stable structure [15]. NSF is a barrel-shaped hexamer, the six subunits comprising the staves of the barrel [14•]. One could easily imagine that the NSF sits over the SNARE pair and uses the hydrolysis of ATP to separate the strands, thereby priming the SNAREs for further rounds of membrane fusion. NSF as an 'unfoldase' would represent a new type of protein topoisomerase, opposite in function to the classical chaperones [16].

An early step in the docking process, before SNARE pairing, is the tethering of vesicles to putative target membranes [17]. p115 is a myosin-shaped molecule implicated in this process [18] and has recently been shown to bind to two Golgi proteins, GM130 and giantin [19•]. GM130 was first identified as part of a detergent-insoluble matrix [20]. The carboxyl terminus binds it tightly to Golgi membranes whereas the amino terminus binds to p115 [19•]. Giantin was first identified using monoclonal anti-Golgi antibodies [21] and sequencing predicted a long rod-like type II membrane protein [22]. Unlike GM130, giantin is found on COPI (coatomer protein I) vesicles and binding studies now suggest that it provides a tethering site for p115 to link these vesicles to GM130 on the membrane (Figure 2) [23•]. p115 binding to Golgi membranes is known to be regulated by phosphorylation, so providing a means of breaking the tethers that form [24•].

GM130 and giantin are members of a growing family of coiled-coil proteins associated with the Golgi apparatus [25,26•,27,28•,29•]. Their predicted rod-like shape and their length suggest that the Golgi and associated vesicles are covered with a filamentous coat that could serve, at least in part, to restrain transport vesicles [17,30,31•]. A COPI vesicle budding from one cisterna could be tethered to the next before budding is complete. The cup shape of most cisternae might even help ensure that the budding vesicle is tethered to the next cisterna rather than to the one before. Tethering would ensure that vesicles are never free to diffuse away from the surface of the Golgi into the surrounding cytoplasm. This would ensure efficient and therefore rapid transport even through multiple layers of stacked cisternae.

The idea that COPI vesicles are never free may even extend to their transport within the early part of the secretory pathway. Rab6 has been implicated in retrograde transport and a Rabkinesin-6 has recently been described that could facilitate this process using the microtubule network [32•]. Other networks might also be involved. There are Golgi homologues of plasma membrane spectrin and ankyrin and recent work implicates them in endoplasmic reticulum (ER)-to-Golgi transport [33,34•,35•]. Myosin II has even been implicated in transport of vesicles from the *trans*-Golgi network (TGN) [36•].

Golgi disassembly

Conditions and agents that disassemble the Golgi apparatus have long been useful in understanding the organisation of this complex organelle. Brefeldin A and ilimaquinone (IQ) are just two of the drugs that have yielded important insights. IQ is a sea sponge metabolite that rapidly and reversibly converts the Golgi apparatus into a collection of small (60–90 nm diameter) vesicles [37] and work during the past year, using permeabilised cells, has shown that this process is inhibited by GTPγS [38•]. This acts not through ADP ribosylation factor (ARF), coatomer or Rab proteins but, interestingly, through heterotrimeric G proteins; furthermore, it is not the G_α subunit that is required but the βγ subunits. These alone, when added to permeabilised cells, fragment the Golgi apparatus in a manner strongly reminiscent of the action of IQ.

Mitosis is the one physiological condition that results in fragmentation of the Golgi apparatus [39•]. Fragmentation is restricted to animal cells [40•] and the stacks of cisternae are replaced by clusters of tubules and vesicles [41]. Two pathways appear to be involved [42]. One is independent of coatomer and so might possibly use the same pathway as that used by IQ. The other depends on coatomer and involves continued budding of COPI vesicles that cannot then fuse. A possible explanation for this inhibition of fusion is provided by recent work showing that the amino terminus of GM130 is phosphorylated under mitotic conditions and this leads to the release of p115 both *in vivo* [39•] and *in vitro* [19•]. If p115 cannot bind, COPI vesicles should not be tethered. If they cannot be tethered, they should not fuse.

The mitotic kinase p34^{cdc2} is involved in the fragmentation of the Golgi apparatus at the onset of mitosis but it operates, at least in part, through other kinases. The most interesting is the MAP kinase pathway which is thought to be activated by the mitotic kinase at the level of MAPK kinase 1 (MEK1) which eventually activates a downstream Golgi-specific extracellular signal regulated kinase (ERK) [43•]. It will be interesting to characterise this ERK and determine which of the known mitotic targets it directly, or indirectly, phosphorylates.

Cisternal reassembly

Golgi fragments generated either by IQ treatment or under mitotic conditions were found to require two distinct fusion ATPases in order to regenerate cisternae [44,45]. One was NSF, the other p97, a protein homologous to NSF but previously of unknown function [46]. The cytosolic form of p97 has a tightly bound cofactor (p47) [47•], unlike NSF. Homologues of p97 are also found in Archae, again unlike NSF, making it the more ancient of the two ATPases [48•]. These and other data implicate p97 in the biogenesis of membranes and NSF in membrane functioning.

Recent work on the reassembly of cisternae from mitotic Golgi fragments has shown that both ATPases compete for a common Golgi t-SNARE, syntaxin 5 [49•]. This syntaxin

is thought to provide the docking site for anterograde vesicles from the ER and retrograde vesicles from later parts of the Golgi, interacting with the appropriate v-SNAREs on these vesicles. As with other syntaxins, syntaxin 5 binds NSF via α-SNAP [50•] and it has now been shown to bind p97 via p47, making p47 analogous in function (if not sequence) to α-SNAP [49•]. p47 competes with α-SNAP for binding to syntaxin 5 and *vice versa*. Each appears to initiate its own fusion pathway and, by so doing, inhibits the other [49•]. Utilising a common t-SNARE might enable the cell to integrate the two functions of NSF and p97 though it is still unclear precisely what these are.

One clue comes from analogous results for the fusion of ER membranes with each other, catalysed by the yeast homologue of p97, CDC48p [51•]. The ER t-SNARE needed for this fusion event is Ufe1p, earlier identified as the t-SNARE involved in the fusion (NSF-mediated) of retrograde vesicles from the Golgi with the ER [52•]. Most importantly, and in marked contrast to NSF, none of the known ER-to-Golgi v-SNAREs are needed for CDC48p to catalyse ER–ER fusion [51•]. Since Ufe1p is needed in both ER membranes that fuse, this suggests that CDC48p might operate on t-t SNARE pairs, just as NSF operates on v-t SNARE pairs. This interpretation is consistent with the observation that the Golgi v-SNARE GOS-28 is needed for NSF- but not p97-mediated reassembly of cisternae from mitotic fragments [49•], though not with the observation that reconstituted liposomes containing only t-SNAREs will not fuse with each other [13•]. More work is clearly needed to determine precisely which SNAREs are needed for ER–ER and Golgi–Golgi fusion but it does suggest that the different functions of NSF and p97 can be ascribed to different SNARE substrates. The heterotypic fusion cycle would utilise v-t SNAREs, the homotypic fusion cycle, t-tSNAREs. For cisternal reassembly, this would mean that p97 reconstitutes the central cores (a homotypic fusion event) whereas NSF would reconstitute the rims (a heterotypic event).

Cisternal stacking

Mitotic Golgi fragments will regenerate cisternae under conditions that prevent their stacking. This observation was used to identify a protein, GRASP65, that is involved in the initiation and/or maintenance of stacked cisternal membranes [53•]. Antibodies to GRASP65 and a recombinant, soluble form of GRASP65 inhibited cisternal stacking, confirming its role as a stacking factor.

GRASP65 is a myristoylated protein, conserved from yeast to mammals, and it forms a tight complex with GM130. Both are heavily phosphorylated during mitosis, suggesting that the inhibition of vesicle docking and cisternal stacking are linked processes. It will clearly be important to understand how this protein complex is targeted to Golgi membranes and how precisely it stacks cisternae. It should also be possible to determine the physiological significance of stacked cisternal structures by disrupting this complex of GRASP65/GM130 *in vivo*.

Conclusions

The central role played by the Golgi apparatus in the transport, modification and sorting of cargo is now well established. The mechanism of transport has been the focus of much recent work and considerable progress has been made in understanding the molecular basis of COPI vesicle budding, docking and SNARE-mediated fusion. This contrasts with the distinct lack of progress in defining the route taken by cargo through the stack, which has led to the resurrection of early transport models including those that postulate nonvesicular mechanisms of transport. We believe that these problems will not be resolved until we understand more about the mechanisms that generate, maintain and regulate the unique architecture of this organelle. Questions need to be focused on: the mechanisms that control the shape, size and number of Golgi cisternae and what significance these have for the functioning of the Golgi apparatus; the regulatory mechanisms that modulate the architecture during interphase when the flux through the Golgi is highest and during mitosis when the architecture is radically changed and cargo transport ceases (at least in animal cells); and, finally, the biogenetic mechanisms that construct new copies of the Golgi apparatus that are inherited by daughter cells during the division process and generate new types of Golgi as cells differentiate during multicellular development.

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