Maturation of Golgi cisternae directly observed

Hugh R.B. Pelham

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

Newly synthesized secretory proteins pass through the Golgi apparatus, which consists of multiple cisternae containing distinct populations of enzymes. Are the cargo proteins shunted between cisternae in vesicles or do they remain in a cisterna while its Golgi enzymes are removed and replaced? As predicted by the latter model – the cisternal maturation hypothesis – two groups have directly observed the replacement of one Golgi protein with another in individual cisternae, thus answering the question. However, its solution raises many more unknowns.

Models for Golgi traffic
Proteins destined for secretion from eukaryotic cells, or for incorporation into the plasma membrane or endosomes, are first inserted into the endoplasmic reticulum. They then undergo transport within membrane carriers to and through the Golgi apparatus, which comprises a stack of apparently discrete cisternae the shapes of which can vary from flat pouches to more fenestrated networks. These cisternae differ in their content of enzymes and other membrane proteins, and it has long been debated how this organization is maintained while cargo proteins are transported through the entire structure. In general, two classes of model have been considered, both drawing on electron micrography (EM) images of vesicular or tubular sections at the rims of cisternae (Figure 1). In the first, cargo proteins travel between fairly static cisternae, either by budding into vesicles that then fuse with the next cisterna [1] or via transient or more permanent tubular connections. In the second, cargo proteins are assumed to remain in the cisternae, while resident Golgi enzymes travel backwards via vesicles or tubules [2]. In this way, a single cisterna matures as its protein content is remodelled, eventually fragmenting into vesicles that leave the Golgi for the plasma membrane or endosomes. Much evidence favours this cisternal maturation model, but controversy remains. For example, the simple models would seem to predict that either cargo or Golgi enzymes should be preferentially incorporated into vesicles, but immuno-EM studies of this have given conflicting results [3–5]. Dramatic support for cisternal maturation now comes from two groups who have directly visualized this process in yeast [6,7].

Watching cisternae mature
Cisternae are, in most cells, too closely stacked together for optical resolution. However, the yeast Saccharomyces cerevisiae has an unusual Golgi structure in that the cisternae are scattered within the cell. Immunofluorescence has shown that cisternae with different marker proteins appear and disappear in rapid 3D motion [8]. It has taken many years to develop green and red fluorescent protein markers that can be expressed at reasonable levels without perturbing the Golgi, and microscopes capable of forming a 3D stack of two-colour fluorescent images every few seconds. Nevertheless, Losev et al. [6] and Matsuura-Tokita et al. [7] can now convincingly follow individual cisternae over periods of several minutes. Losev et al. [6] show that integral membrane marker proteins appear and disappear in principle, be observed in real time in a live yeast cell.
individual cisternae, with lifetimes of approximately two minutes. The peripheral protein Sec7 shows similar kinetics, which is consistent with a coordinated change in membrane identity. Most importantly, markers of the early Golgi are consistently replaced with late Golgi markers (but never vice versa) (see Figure 2). Together, the groups have studied six different markers. In addition, Matsuura-Tokita et al. [7] can distinguish two different transitions: (i) from early cisternae marked with Rer1 to those containing the medial Golgi protein Gos1, and (ii) from these to late cisternae that are Sec7-positive. Intermediate forms with two markers can be observed, and processing of the images suggests that the individual cisternae are composed of small networks of structures that can individually change identity [7]. Finally, a mutant allele (ret1–1) of coat protein complex I (COPI) that is thought to control inter-cisternal traffic slows the maturation process [7], indicating that the observed changes are indeed driven by the normal transport machinery.

These results are spectacular and seem to be the definitive proof of cisternal maturation in yeast and, by inference (because the membrane traffic machinery is generally well conserved), in other cells also. However, although confirmation of the basic phenomenon is a milestone, it draws attention to the many remaining uncertainties and disputes.

Where is the cargo?
The simple model assumes that cargo proteins invariably remain within the cisterna (Figure 2). In animal cells, although some EM evidence supports this [9], kinetic studies with fluorescent proteins have suggested that cargo proteins can spread between cisternae to form a single, well-mixed pool either via tubular connections or by bi-directional vesicular traffic [10]. This would enable some cargo molecules to traverse the Golgi more rapidly than the cisternae mature. However, the average transport rate for cargo would still be determined by the rate of net membrane efflux from the Golgi. Thus, although the observed broad correlation between transport and

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**Figure 1.** Models for Golgi traffic. Two simple models have been proposed. (a) In the stable cisternae model, cargo (represented by asterisks) is transported in vesicles between stable structures that differ in their enzyme content (represented by different colours). (b) In the cisternal maturation model, the cargo remains within the cisternal structure and the enzymes are transported backwards in vesicles. Cisternae are, thus, remodelled around the cargo. Flow is maintained by the creation of new cisternae at the beginning of the Golgi and their disintegration at the far side. Variations of these models include transient or stable direct connections between cisternae, bi-directional vesicular traffic, and combinations of maturation and vesicular transport.

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**Figure 2.** Experimental observation of cisternal maturation. Representation of the experimentally observed time course of the maturation of a greatly simplified Golgi stack, depicted in the first instance as two cisternae. To identify late cisternae, a late Golgi membrane protein has been tagged with a red fluorescent marker; to identify an early cisterna, an early Golgi membrane protein has been tagged with a green fluorescent protein. (a) During the time course of the experimental visualization, the late cisterna disappears as the red marker is extracted and transported back to the early cisterna (a process represented by arrow 1). (b) The late cisterna, presumably, fragments into transport vesicles destined for the plasma membrane (arrow 2). When the early cisterna receives the red marker it transiently appears yellow owing to the mixing of the fluorescent signal from the green and red markers. Detailed studies show that this change can occur sequentially in sub-structures of a single network-like cisterna [7]. The green protein is then lost to a newly formed cisterna (arrow 3). (c) Transformation into a late cisterna is completed and the cycle begins again. Concomitantly, new cisternae are formed from ER-derived vesicles and become visible as they receive the green marker (arrows 3 and 4), which becomes the green, newly formed cisterna in (c).

Cargo proteins (asterisks) were not visualized by the techniques used in the experiments of Losev et al. [6] and Matsuura-Tokita et al. [7] but are thought to remain in the cisterna during the course of its maturation (and thus end the process in what has become a late cisterna having started in what was an early cisterna). The transport intermediates, whether vesicles or narrow tubules, are also too small and faint to be seen clearly by these techniques [6,7] but, for simplicity, they have been depicted as vesicles here. It is the change in colour, and hence protein composition, of single continuously tracked cisternae that is the hallmark of cisternal maturation and the key observation in these experiments.
cisternal maturation rates [6] is consistent with the static cargo model, it does not distinguish it from one in which cargo is mobile and the rate of membrane efflux from the Golgi is determined by maturation. Direct observation of cargo would resolve this issue, but will not be easy.

What defines a cisterna?
A major question raised by the recent observations is that of how the size of a cisterna is maintained. Cargo proteins form a minor proportion of the membrane and, if resident proteins can come and go, it is not obvious what constitutes the core or how cisternae avoid complete collapse. One possibility is that lipids have a role. Sphingolipids are synthesized and associate with sterols in the Golgi, both in animal cells [11] and in yeast [12], and their accumulation results in a thickening of the membrane bilayer. Sphingolipid synthesis in maturing cisternae would, thus, generate a gradient of lipid composition through the Golgi, provided that sphingolipids themselves could not pass easily between cisternae. Golgi enzymes, the transmembrane domains of which are shorter than those of plasma-membrane proteins and thus are incompatible with a sterol-rich bilayer, would partition within this gradient according to their physical properties [11–13]. The key assumption of this model is that sphingolipids do not easily enter the carriers that mediate traffic between cisternae and, if this is the case, these lipids could provide the core structure that maintains the existence of a cisterna. It would, therefore, be interesting to know whether traffic of sphingolipids and sterols between cisternae is indeed slow.

A related question concerns the mechanism of transport between cisternae. Initial studies in yeast were consistent with vesicular transport, and showed a requirement for membrane fusion mediated by specific SNARE (soluble NSF-attachment receptor) proteins [8]. Thus, a temperature-sensitive mutation in Srt1, a SNARE required for intra-Golgi transport, resulted in rapid accumulation of a late Golgi marker in vesicles while early Golgi cisternae, both permeable to cargo molecules. The vesicular model is that it avoids connecting dissimilar cisternae directly together, which might be expected to result in mixing of all components. However, there is increasing evidence that domains of distinct protein composition can form and be maintained even within a continuous membrane. The Golgi might thus be thought of as a series of domains, perhaps with coat proteins that induce vesicles and narrow tubules, which would serve to filter the traffic of components between them. Segregation of proteins in this manner is likely to be more fundamental to Golgi function than properties such as overall shape and topological structure, which differ substantially between cell types and species.

A striking example of domain formation comes from recent studies of the yeast plasma membrane [15]. This indisputably intact structure shows clear and stable segregation into a domain containing the plasma membrane ATPase, punctuated by regions populated by amino acid transporters and other proteins, from which the ATPase is excluded. Segregation depends on sterols and seems to correlate with sterol distribution. Other proteins, such as SNAREs, are uniformly distributed and can evidently penetrate both kinds of domain [16]. The physical basis for this phenomenon is not understood, but it suggests an intriguing analogy with the Golgi, with the domains representing different cisternae, both permeable to cargo molecules. The added twist with the Golgi, of course, is that we now know that a single structure undergoes sequential identity changes, and we need to understand not only the static biophysical properties of the membranes but also their dynamics.

Concluding remarks
As the Golgi slowly reveals its secrets, it is apparent that much of the uncertainty about its workings stems from our imperfect understanding of the properties of biological membranes, with their complex mixtures of lipids and integral and peripheral proteins, and this is still a challenge for the future. There is nothing like direct visualization to make a model real but, as always, one answer stimulates many questions.

Membrane domains
The distinction between vesicular and tubular transport might be less significant than it seems. The attraction of the vesicular model is that it avoids connecting dissimilar cisternae directly together, which might be expected to result in mixing of all components. However, there is increasing evidence that domains of distinct protein composition can form and be maintained even within a continuous membrane. The Golgi might thus be thought of as a series of domains, perhaps with coat proteins that induce vesicles and narrow tubules, which would serve to filter the traffic of components between them. Segregation of proteins in this manner is likely to be more fundamental to Golgi function than properties such as overall shape and topological structure, which differ substantially between cell types and species.

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References
Structure of the Ctr1 copper trans‘PORE’ter reveals novel architecture

Yasuhiro Nose, Erin M. Rees and Dennis J. Thiele

Department of Pharmacology and Cancer Biology, Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Research Drive-LSRC C351, Durham, NC 27710-3813, USA

Copper is essential for biological processes such as free radical detoxification, mitochondrial respiration and iron metabolism. A central player in copper homeostasis is the high-affinity integral plasma membrane copper transporter Ctr1. However, the precise mechanisms by which Ctr1 functions are not known. Here, we highlight an important breakthrough in our understanding of how Ctr1 facilitates Cu(I) movement across membranes: the publication of structural details for human Ctr1 obtained from 2D crystallography and electron microscopy.

Eukaryotic copper transporter
Copper is an essential nutrient for almost all eukaryotic organisms to carry out biological processes such as free radical detoxification, mitochondrial respiration and iron metabolism. A central player in copper homeostasis is the high-affinity integral plasma membrane copper transporter Ctr1. However, the precise mechanisms by which Ctr1 functions are not known. Here, we highlight an important breakthrough in our understanding of how Ctr1 facilitates Cu(I) movement across membranes: the publication of structural details for human Ctr1 obtained from 2D crystallography and electron microscopy. putative pore between the subunit interfaces (Figure 1), suggesting that this family of copper transporters is structurally similar to channel proteins. Furthermore, the data address fundamentally crucial aspects of the mechanisms by which Ctr1 transports Cu(I) across the plasma membrane to the intracellular delivery pathway [5].

Biological and physiological relevance of Ctr1
Ctr1 is a member of a family of proteins that provide copper to the copper chaperones, which then traffic copper ions to specific intracellular proteins or compartments such as the secretory machinery, mitochondria or Cu, Zn superoxide dismutase. The importance of Ctr1, and by inference its role in copper uptake, in mammalian growth and development was demonstrated in two independent gene-targeting studies in mice [6,7]. Indeed, homozygous deletion of Ctr1 resulted in embryonic lethality and a range of developmental phenotypes, with Ctr1−/− embryos ultimately resorbed into the uterus mid-way through gestation. Importantly, whereas Ctr1+/− mice exhibited no outward growth or developmental defects, they displayed copper accumulation defects in a subset of tissues including brain and spleen. Mouse embryonic fibroblasts lacking Ctr1 are largely defective in high-affinity copper uptake, demonstrating a prominent physiological role for Ctr1 in copper accumulation [8]. Furthermore, studies have suggested a role for Ctr1 in the accumulation of cisplatin, a potent and effective anti-cancer agent [9].

Ctr1 mechanism of action
Although the precise mechanisms by which Ctr1 enhances copper movement across the plasma membrane are not known, recent studies have provided a basis for a working model. First, Ctr1 acts on reduced copper [Cu(I)] rather than the oxidized metal ion, Cu(II). This preference for Cu(I) is supported by the requirement of yeast cell-surface

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