Hypothesis

A cisternal maturation mechanism can explain the asymmetry of the Golgi stack

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Abstract Morphological data suggest that Golgi cisternae form at the *cis*-face of the stack and then progressively mature into *trans*-cisternae. However, other studies indicate that COPI vesicles transport material between Golgi cisternae. These two observations can be reconciled by assuming that cisternae carry secretory cargo through the stack in the anterograde direction, while COPI vesicles transport Golgi enzymes in the retrograde direction. This model provides a mechanism for cisternal maturation. If Golgi enzymes compete with one another for packaging into COPI vesicles, we can account for the asymmetric distribution of enzymes across the stack.

Key words: Golgi apparatus; Coated vesicle; Cell compartmentation; Intracellular transport; Protein sorting; Secretory pathway

1. Introduction

Secretory proteins move from the ER to the Golgi apparatus, and then transit through the Golgi stack in the *cis*-to-*trans* direction before being sorted into different types of vesicles at the *trans*-Golgi network (TGN) [1,2]. Early electron microscopy studies suggested that the Golgi operates by cisternal maturation. In this view, secretory proteins collect in a newly-forming cisterna at the *cis*-face of the stack; this cisterna would then migrate through the stack, somehow maturing in the process [3]. However, later investigations revealed that Golgi stacks are asymmetric, with some resident enzymes concentrated in *cis*-cisternae and others concentrated in *medial*- or *trans*-cisternae [1,4]. These observations were not readily explained by the maturation model. As an alternative it was proposed that the Golgi consists of a series of stable subcompartments [1,4]. Secretory cargo would be carried from one subcompartment to the next in transport vesicles, while resident Golgi enzymes would be retained within the cisternae [5–7]. In support of this hypothesis, Rothman and colleagues used a cell fusion assay to demonstrate that a secretory protein can transfer between Golgi stacks [8]. These results were complicated by the finding that *trans*-Golgi elements in different stacks rapidly form interconnections [9], but further evidence for vesicular transfer came from a cell-free system [10]. Analysis of this system indicated that intra-Golgi transport is mediated by COPI vesicles, and that fusion of COPI vesicles with cisternae requires the generalized NSF/SNAP/SNARE machinery [10–12].

Despite the apparent success of the stable subcompartments model, it does not fully account for the experimental data [2,3]. For example, in certain algae, large glycoprotein 'scales' are synthesized in the Golgi and then transported to the cell surface; these scales can be detected only within Golgi cisternae and never in the associated transport vesicles [13]. Similar observations were made with mammalian cells that transport lipoprotein particles, casein submicelles or virus particles through the Golgi stack [14–16]. Other studies indicated that Golgi enzymes are not segregated into precisely defined subcompartments, but instead form overlapping concentration gradients within the stack [17–20]. Recent work has revealed surprising properties of Golgi enzymes: they are highly mobile within the cisternal membranes [21], they can be packaged into COPI vesicles [22–24], and they can travel between different cisternae [25–27]. Such considerations have prompted renewed interest in cisternal maturation and indeed, some morphologists have never abandoned this notion [3,13–16,20,28–30].

Fig. 1 shows an updated version of the maturation model. The added feature is that COPI vesicles are postulated to transport Golgi enzymes in the retrograde direction as the cisternae migrate in the anterograde direction [20,29,31,32]. A new cisterna would form as follows: ER-derived COPII vesicles [33] fuse with one another and with retrograde COPI vesicles carrying *cis*-Golgi enzymes, and other COPI vesicles retrieve selected membrane components back to the ER [20,23,30–32,34,35]. Each cisterna would mature by donating one set of Golgi enzymes to the adjacent 'younger' cisterna, while receiving a different set of enzymes from the adjacent 'older' cisterna.

The maturation idea profoundly affects both the formulation of questions about the Golgi and the interpretation of experimental findings. Because this model views the Golgi as a unitary organelle rather than a series of distinct compartments, it explains why so few components have been specifically implicated in transport through the Golgi stack [36], and it eliminates the problem of defining the number of Golgi subcompartments and the number of cisternae per subcompartment [1,2]. This model makes the following predictions, all of which accord with experimental evidence:

1. Large structures should be capable of transiting through the stack, and Golgi enzymes should be mobile and present in COPI vesicles (see above).
2. The processing of secretory proteins by Golgi enzymes should require retrograde transport of the enzymes, and

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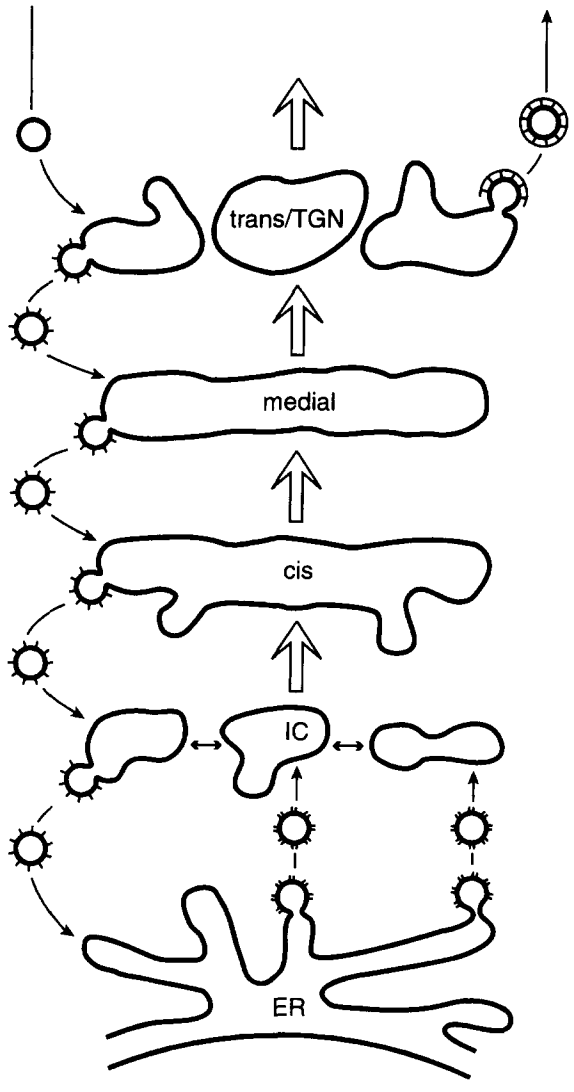

Fig. 1. A cisternal maturation model. The ER-Golgi intermediate compartment (IC) represents a nascent cisterna that forms as ER-derived COPII vesicles fuse with one another and with retrograde COPI vesicles from the *cis*-Golgi. Other COPI vesicles recycle material from the IC back to the ER. The new *cis*-cisterna migrates through the stack, gradually acquiring *medial*- and *trans*-characteristics. This maturation is effected by retrograde COPI vesicles, which carry resident Golgi enzymes between cisternae. At the *trans*-Golgi network (TGN) stage, the cisterna produces various types of coated vesicles [35,39] and ultimately evolves into a collection of secretory vesicles. Additional vesicles retrieve membrane from post-Golgi compartments to the TGN [7].

should therefore involve COPI vesicles and the NSF/SNAP/SNARE machinery. Indeed, numerous studies have implicated these components in intra-Golgi transport [10]. Although such data have been taken as evidence that COPI vesicles transport secretory cargo between cisternae, it is equally possible that COPI vesicles function to carry Golgi enzymes. A recent analysis of the cell-free intra-Golgi transport system supports this revised interpretation (J. Ostermann, personal communication).

3. For the reaction traditionally described as 'ER-to-Golgi transport', both COPI and COPII vesicles should be needed [35]. The reason is that secretory proteins would leave the ER in anterograde COPII vesicles, and *cis*-Golgi enzymes would travel to the newly-forming cisterna in retrograde COPI vesicles.
4. Constitutive secretory vesicles should arise from the TGN not by a classical budding process, but rather by the selective removal of material targeted to other destinations [37]. Tubular elements of the TGN would be consumed by the formation of coated vesicles, and the remaining globular elements would become secretory vesicles [38,39]. This interpretation may explain why no coat proteins have been conclusively implicated in TGN-to-plasma membrane transport [35].

The present analysis concerns Golgi asymmetry, the issue that led to the downfall of the original maturation idea. We have focused specifically on type II enzymes of the Golgi stack [5]. The challenge was to determine whether a maturation mechanism could result in various enzymes being concentrated in *cis*-, *medial*- or *trans*-cisternae. It turns out that a simple extension of the maturation model can generate this pattern.

2. A cisternal maturation mechanism

Based on the scheme outlined in Fig. 1, we formulated a quantitative model that incorporates the following definitions and assumptions:

1. In a single 'cycle', each cisterna advances one step in the anterograde direction. During this cycle the *trans*-most cisterna loses all of its Golgi enzymes and matures into secretory vesicles. Meanwhile, a new cisterna forms at the *cis*-face of the stack.
2. Part of the membrane of the new cisterna derives from retrograde COPI vesicles, and the remainder is provided by net forward transport of membrane from the ER. The amount of net forward transport will be low in non-growing cells but higher in rapidly growing cells. For convenience, we assume that retrograde transport contributes ~50% of the membrane of the new cisterna (although the model works equally well for other percentages). Therefore, during one cycle, retrograde COPI vesicles carry the membrane equivalent of about half of a cisterna between each cisterna in the stack.
3. The total population of Golgi enzymes is carried backwards in COPI vesicles by one cisterna (on average) during each cycle. Thus, based on assumption (ii), the average density of Golgi enzyme molecules in COPI vesicles will be twice the average density in the stack of cisternae.

This model implies that the cell has a regulatory system for ensuring that cisternal progression is balanced by retrograde vesicular transport of lipids and Golgi enzymes. In support of this notion, Golgi structure appears to be maintained by a dynamic balance of membrane flow [3,30].

It is not difficult to explain how a particular Golgi enzyme could end up concentrated near the *cis*- or *trans*-face of the stack: retrograde vesicular transport of this enzyme would be faster or slower, respectively, than cisternal progression. More problematic is the finding that some enzymes are concentrated in *medial*-cisternae [4]. As shown below, this pattern will be

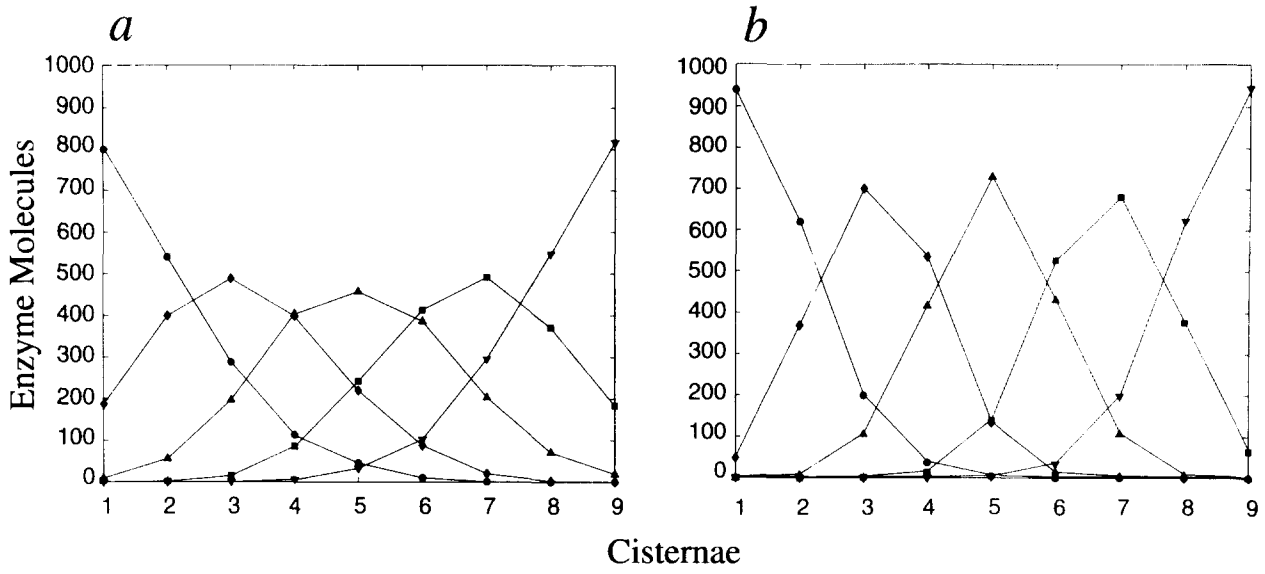

Fig. 2. Simulated distribution of Golgi enzymes after repeated cycles of cisternal progression/maturation. The simulation was performed as follows. Let n_c = the number of cisternae, n_s = the number of enzyme species, n_t = the total number of enzyme molecules, and n_v = the number of vesicles required to equal the membrane area of one cisterna. Here $n_c = 9$, $n_s = 5$, $n_t = 9000$, $n_v = 200$; i.e. five enzyme species are present at 1800 molecules each in a stack of nine cisternae. Our model assumes that during a single cycle of Golgi operation, each cisterna produces an average of $n_v/2$ vesicles carrying n_t/n_c Golgi enzyme molecules, so each vesicle carries $2n_t/n_c n_v$ enzyme molecules. (These precise assumptions can be relaxed without qualitatively altering the results.) During the simulation, a vesicle buds from a randomly chosen cisterna and fuses with the adjacent 'younger' cisterna. The enzyme content of the budding vesicle is determined stochastically using a weighted average: if n_i is the number of enzyme molecules of species i in the selected cisterna, the probability of choosing species i is given by $p(i) = a_i n_i / \Sigma_{j=1}^{n_s} a_j n_j$. The a_i values (weighting factors) are chosen from a Boltzmann distribution that reflects the relative potential energies for packaging the different enzymes into the vesicle: $a_i = e^{\lambda(i-1)}$. Thus, each successive enzyme in the series A (●), B (♦), C (▲), D (■), E (▼) is favored over its predecessor by a factor of e^{λ}. Results are shown for λ values of (a) 1.0, and (b) 2.5. As soon as the *trans*-most cisterna is entirely depleted of Golgi enzymes, the remaining cisternae are advanced by one step and a new *cis*-cisterna begins to form. This transition is defined as the completion of a cycle. Each simulation involves between 100 000–150 000 budding events (~150–200 cycles). After the system has reached a steady state, the number of enzyme molecules per cisterna is recorded at the end of each cycle, and these numbers are averaged over 100–150 cycles.

generated if Golgi enzymes compete with one another for packaging into COPI vesicles.

3. Sorting by competition

Suppose that the rate of retrograde enzyme transport is limited by the carrying capacity of COPI vesicles. The result will be a competition, in which various Golgi enzymes are packaged into vesicles with different relative efficiencies. The packaging efficiencies might reflect different binding affinities of the enzymes for a receptor, or different tendencies of the enzymes to partition into the vesicle membrane [6].

With this competition mechanism, the strongest competitors are *cis*-Golgi enzymes, which will be recycled rapidly from every cisterna. The intermediate competitors are *medial*-Golgi enzymes, which will be recycled rapidly from cisternae that are depleted of *cis*-Golgi enzymes. The weakest competitors are *trans*-Golgi enzymes, which will only be recycled rapidly from cisternae that are depleted of both *cis*- and *medial*-Golgi enzymes.

Fig. 2 shows the results of a simulation in which five enzymes labeled A–E are present in equimolar amounts in a stack of nine cisternae. Enzyme A is packaged into retrograde vesicles more efficiently than enzyme B, which is packaged more efficiently than enzyme C, etc. In Fig. 2a the packaging energies of the various enzymes are only slightly different, and the result is a series of broad overlapping peaks of enzyme concentration. In Fig. 2b the differences in packaging energies are greater and the peaks are correspondingly sharper. These results confirm that the competition mechanism can generate distinct peaks of enzyme concentration with varying degrees of overlap. In general, the separation between any two enzymes will be a function of their relative packaging energies.

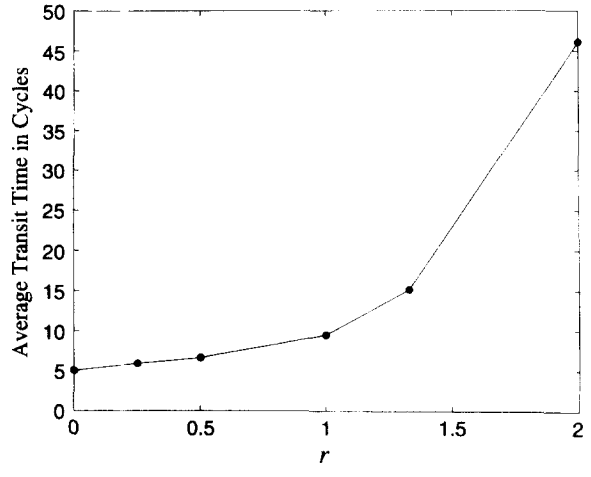

Fig. 3. Transit of a secretory protein through a stack of five cisternae. At the beginning of each simulation, a bolus of 750 secretory protein molecules is deposited in the *cis*-most cisterna. Repeated cycles of cisternal progression/maturation are then simulated as in Fig. 2, except using $n_c = 5$, $n_s = 3$, $n_t = 3750$, $n_v = 300$. During a budding event, each secretory protein molecule in the cisterna has a probability of r/n_v of being incorporated into the vesicle. The average transit time is computed by repeatedly 'tagging' one molecule of the secretory protein and tracking its progress. This molecule is considered to have transited the stack if it remains in the *trans*-most cisterna after this cisterna has been depleted of Golgi enzymes.

Our simulations were performed using a simple set of parameters. To make sure that the observed asymmetry does not depend upon an idealized representation of the system, we tested the model under conditions in which vesicles could either (i) occasionally travel by more than one cisterna in the retrograde direction, or (ii) occasionally fuse with the adjacent cisterna in the anterograde direction. Both conditions still yielded asymmetric enzyme distributions, although the concentration peaks were broader than in Fig. 2. Interestingly, if more than ~15% of the vesicles traveled back by two cisternae instead of one, enzyme distributions were destabilized and the asymmetry was lost. Thus, our maturation model will generate asymmetric enzyme distributions if most of the vesicles travel by one cisterna in the retrograde direction, and if a competition mechanism biases the recycling of the enzymes.

4. Movement of Golgi enzymes within the stack

Because the packaging of Golgi enzymes into COPI vesicles is a stochastic process, individual enzyme molecules will not be restricted to a particular position in the stack. For example, if an enzyme species is concentrated in *cis*-cisternae, any given molecule of this enzyme will occasionally reach *medial*- and *trans*-cisternae. We simulated this effect as follows. A stack of five cisternae contained a *cis*-, a *medial*- and a *trans*-Golgi enzyme. Computations revealed that an average of 20–75 cycles were needed for a given molecule of the *cis*-Golgi enzyme to reach the *trans*-most cisterna, or for a given molecule of the *trans*-Golgi enzyme to reach the *cis*-most cisterna. How do these numbers compare with experimental observations? It has been estimated that each cycle of cisternal progression takes 1–3 min [3]. Various Golgi enzymes apparently 'wander' to distant cisternae with half-times ranging from several minutes to several hours [25–27], corresponding to ~5–100 cycles. The computed values are in this range, indicating that our model can plausibly account for the movement of Golgi enzymes within the stack.

5. Transit of secretory proteins through the Golgi stack

A protein that is excluded from COPI vesicles will transit through the stack at the rate of cisternal progression. For example, electron microscopy studies suggest that certain kinds of secretory cargo are absent from COPI vesicles, or are much less concentrated in these vesicles than in Golgi cisternae [13–15] (J. Bergeron, personal communication). However, some secretory proteins can be packaged into COPI vesicles [11,12,22,24]. In the maturation model, this retrograde vesicular traffic will slow the advance of the secretory protein through the stack. We can quantify this effect as follows. For a given secretory protein, define r as the average ratio of the concentration of the secretory protein in COPI vesicles to the concentration in the parental cisternae: $r = 0$ corresponds to complete exclusion of the protein from COPI vesicles, and $r = 1$ corresponds to packaging of the protein into COPI vesicles at the prevailing bulk concentration. Fig. 3 shows the average transit time of a secretory protein through a stack of five cisternae as a function of r. The transit time increases from 5 cycles at $r = 0$ to 46 cycles at $r = 2$, but at $r = 1$ the transit time is only 9.5 cycles. Thus, secretory proteins that are packaged into COPI vesicles may still advance through the stack quite rapidly.

6. Summary and outlook

We have described a cisternal maturation mechanism that could generate enzyme distributions similar to those seen in actual Golgi stacks. A given Golgi enzyme will be concentrated in the part of the stack at which retrograde vesicular transport of this enzyme balances cisternal progression. In this scenario, different Golgi enzymes compete for a limited number of sites in budding COPI vesicles. The enzymes that are the strongest competitors will be concentrated near the *cis*-face; enzymes with intermediate competitive ability will be concentrated in *medial*-cisternae; and the enzymes that are the weakest competitors will be concentrated near the *trans*-face (Fig. 2). An essential feature of this mechanism is that for each enzyme species, the probability of being packaged into COPI vesicles increases in the *cis*-to-*trans* direction. The same effect might be achieved by a process other than competition [6]. Regardless of the precise mechanism, we have demonstrated that cisternal maturation is compatible with Golgi asymmetry.

Our model shares features with an expanded version of the 'distillation tower' hypothesis [10]: in both cases, Golgi enzyme distributions would be maintained by a stochastic balance of anterograde and retrograde transport. However, we suggest that the anterograde carriers are whole cisternae rather than COPI vesicles, and that such vesicles function solely as retrograde carriers. The proposed maturation mechanism requires that most of the vesicles budding from one cisterna fuse with the adjacent cisterna in the retrograde direction. There is strong evidence for retrograde traffic from all stages of the Golgi stack [7,10,26,27,31], but we still do not know how COPI vesicles might be targeted from each cisterna to the neighboring 'younger' cisterna. Although vesicle fusion presumably involves the NSF/SNAP/SNARE machinery, it is unlikely that the specificity of COPI vesicle targeting depends upon multiple different SNARE proteins because the maturation concept implies that intra-Golgi transport is a homotypic process. One possibility is that COPI vesicles move in the retrograde direction by tracking along an oriented filamentous scaffold [40]. The mechanism of retrograde vesicle targeting emerges as the central question raised by our model.

A consequence of the maturation hypothesis is that Golgi enzyme distributions will take the form of concentration gradients rather than absolute separations (Fig. 2). Moreover, individual enzyme molecules would be expected to visit different cisternae in the stack. Both of these predictions fit the experimental data [17–19,25–27]. Our model makes two additional predictions. First, the density of type II Golgi enzymes should be at least as high in COPI vesicles as in the cisternal membranes. Second, the packaging of a secretory protein into COPI vesicles should not promote forward transport, as previously assumed, but should actually retard transit of the protein through the stack (Fig. 3). Hence, experimental tests of this model will center around the nature of the cargo present in COPI vesicles. One careful study found that type II Golgi enzymes were less concentrated in COPI vesicles than in the cisternae [24]. However, those vesicles were formed in vitro in the presence of GTPγS, and it will be important to

describe the contents of COPI vesicles during normal Golgi function in vivo.

Whereas the stable subcompartments model treats the Golgi as an independent organelle, the maturation model implies that the Golgi is a dynamic outgrowth of the ER. Thus, the transitional ER [41] and the ER-Golgi intermediate compartment [23,30,31,42] can be thought of as the first and second cisternae in the stack. In this view, a given protein might show a steady-state concentration in the ER, the intermediate compartment or the Golgi, depending upon how efficiently the protein is retrieved from different locations [7,31]. Another consequence of the maturation model is that if a new transitional ER site were created, vesicles budding from this site would coalesce into cisternae – i.e. Golgi stacks could form de novo from the ER [3,43].

Cell biologists are still attempting to define the basic principles that underlie the Golgi function. Our analysis suggests that an updated version of the maturation idea may provide a useful paradigm. By proposing vesicular transport as the mechanism of cisternal maturation, it is possible to incorporate the major biochemical, morphological and genetic data into a coherent model.

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