CELL BIOLOGY

# The Golgi grows up

Vivek Malhotra and Satyajit Mayor

**The Golgi apparatus of the cell has long baffled biologists, mainly because it is unclear how proteins are conveyed through it on their way to the cell surface. Some innovative microscopy may resolve the issue.**

The Golgi apparatus is the enigmatic organelle responsible for modifying newly synthesized proteins that are destined to be secreted from the cell. Discovered by Camillo Golgi in 1898, the apparatus consists of a stack of disc-shaped membranes called cisternae. Newly made secretory proteins are delivered to the *cis* face of the Golgi. They exit, fully processed, at the opposite end — the *trans* face. But how do the proteins traverse the Golgi stack? Debate on this hotly contested question has persisted, in one form or another, for about 40 years. Now, two papers in this issue[1,2] claim to resolve the matter in favour of one of the two competing transport mechanisms — the so-called 'cisternal maturation model'.

This model proposes that the cisternae progress through the Golgi, gradually moving through the stack as new layers form at the *cis* face and old layers disperse from the *trans* face, and that they carry the secretory proteins with them (Fig. 1a). By contrast, the 'vesicle-shuttle model' (Fig. 1b) proposes that Golgi cisternae are long-lived structures, with secretory proteins being transported from layer to layer in small bubble-like membrane vesicles, which bud off one cisterna and fuse with the next one to disgorge their protein cargo.

At the heart of the controversy are the transport vesicles called COPI vesicles — after the coat-protein complex I (COPI) proteins that encrust their surface. There is a broad consensus that COPI vesicles bud from Golgi cisternae[3], but no clear evidence about what these vesicles carry, or in which direction they travel — *cis* to *trans*, or the reverse[4]. In yeast, some secretory proteins can be secreted in the absence of COPI function, suggesting that there must be another mechanism for their transport[5]. Furthermore, algae secrete large sugar–protein conjugates (called scales), which are processed in the Golgi and can be 20 times the size of a COPI vesicle[6]. These observations argue in favour of the Golgi cisternae carrying the material forward, as proposed in the cisternal maturation model.

But what then do the COPI vesicles do? One proposal is that they carry Golgi proteins in the retrograde direction, recycling resident Golgi proteins from cisternae that are fragmenting at the *trans* face and incorporating them into new layers at the *cis* face. In this scheme, the cisternae break up at the *trans* face to make COPI vesicles, as well as the secretory vesicles that carry secretory proteins for the final step of their journey. The proteins end up either at the plasma membrane, where they are expelled from the cell, or at an organelle called an endosome. This version of the cisternal maturation model received a boost when Bonfanti *et al.*[7] showed that 300-nm procollagen bundles, which are eventually secreted, travel forward through mammalian Golgi stacks without leaving the cisternae.

How do proponents of the vesicle-shuttle model interpret these results? Orci and colleagues[8] proposed that bulky secretory cargoes are transported by 'mega-vesicles' that can be substantially larger than conventional COPI vesicles. This is not impossible. The kinetics of membrane fission (the mechanism by which a budding vesicle separates from the membrane on which it forms) might regulate the size of a vesicle, so that bulkier cargoes delay the final fission event until a vesicle of suitable size has been generated[9]. Another argument against cisternal maturation is that, contrary to its predictions, several studies report that resident Golgi proteins are not concentrated in COPI vesicles[4].

These unresolved issues meant that the cisternal maturation concept remained unproved. But, just as it looked as if the debate would drag on for ages yet, Losev *et al.* (page 1002)[1] and Matsuura *et al.* (page 1007)[2] have independently produced striking images that purport to show direct evidence for cisternal maturation.

The authors exploited the fact that in the budding yeast *Saccharomyces cerevisiae*, the Golgi cisternae are not stacked. This organization makes it possible to follow cisternal dynamics by video microscopy of live cells. Resident proteins characteristic of early and late Golgi cisternae were tagged with two differently coloured fluorescent tags to mark the different cisternae (for example, green for early cisternae and red for late ones). Remarkably, the tagged cisternae changed colour over time, showing a consistent progression. For instance, a green early Golgi cisterna became gradually yellow and then red, as it lost the early Golgi marker and acquired the late Golgi marker. (In such imaging studies, the presence of both green and red fluorescence gives a yellow colour.) These findings are consistent with a cisternal maturation model for Golgi trafficking in budding yeast.

The story is not yet complete: a few key predictions of the cisternal maturation model remain to be tested. For instance, secretory cargoes should remain within the cisternae as they mature, while the resident Golgi proteins come and go — the obvious experiment to confirm this would be to fluorescently tag secretory cargoes at the same time as resident Golgi proteins and watch their relative progress.

Another prediction is that secretory cargoes present in an early cisterna should move through the Golgi together. Preliminary evidence is that they do: Losev *et al.*[1] report that two yeast secretory cargoes (α-factor and carboxypeptidase Y) traverse the Golgi at roughly the same rate. Moreover, Mironov *et al.*[10] showed that two mammalian secretory cargoes, procollagen and the vesicular stomatitis virus glycoprotein (VSV-G), travel through the Golgi at approximately the same speed. But VSV-G at high concentrations

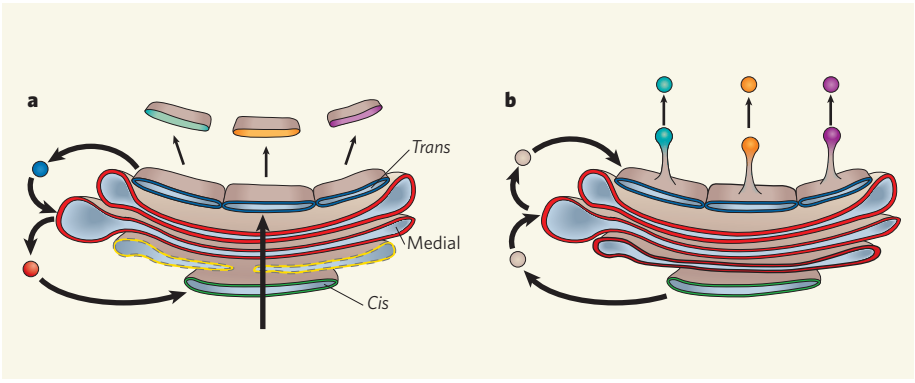


**Figure 1 | Two models of protein trafficking through the Golgi. a,** In the cisternal maturation mechanism, vesicles derived from the endoplasmic reticulum fuse to form the *cis* cisternae of the Golgi (green). This cisterna then transforms into the medial cisterna (outlined in red) by a maturation process, possibly via an intermediate (yellow). Finally, the medial cisterna matures into the *trans* face of the Golgi (blue), to be consumed during secretory traffic to the cell surface. Sorting proteins for different destinations is accomplished by dividing cargo into different regions of the *trans* compartment of the Golgi before it fragments to generate vesicles for different destinations. COPI vesicles would retrieve and recycle resident Golgi proteins at each step. **b,** In the vesicle-shuttle mechanism, the *cis*, medial and *trans* cisternae of the Golgi remain in place, without maturing, and COPI vesicles deliver cargo between cisternae. At the *trans* compartment, vesicles containing different secretory cargoes bud off to deliver their contents to distinct destinations.

might make large protein complexes, and therefore behave as a large cargo, so additional experiments are needed to look at smaller cargoes in mammalian cells.

The fate of the late cisternae also needs to be clarified. If the cisternal maturation model is correct, they should mature into secretory vesicles and other types of carrier, but this has yet to be confirmed by fluorescence microscopy. A related prediction is that blocking retrograde traffic should block cisternal maturation and send Golgi-resident proteins to the cell surface or the endosome. Surprisingly, Matsuura *et al.*[2] find that in a yeast mutant with a defect in COPI vesicle assembly, cisternal maturation is slowed about threefold — but it still occurs. How are resident Golgi proteins being recycled in the absence of COPI function? Do resident Golgi proteins escape from the organelle under these conditions?

Even if cisternal maturation does occur, the details of the mechanism have yet to be defined. For instance, how is the polarized *cis*-to-*trans* distribution of resident Golgi proteins maintained? One suggestion is that it might be by the differential recycling of the Golgi proteins[4]. And what of other cell types? It is quite possible that in *S. cerevisiae*, the Golgi is formed *de novo* and then consumed during each round of secretory protein transport. In mammalian cells, however, there is no evidence that Golgi membranes form *de novo* under physiologically relevant conditions during sequential rounds of secretion. Maybe these cells rely instead on a variety of trafficking modes commensurate with cargo size and Golgi organization. Whatever the answer may be, the images provided by Losev *et al.*[1] and Matsuura *et al.*[2] have certainly swung the scales heavily towards cisternal maturation, in yeast at least. ■

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PLANT BIOLOGY

# Designs on Rubisco

Howard Griffiths

**Rubisco is said to be both the most important enzyme on Earth and surprisingly inefficient. Yet an understanding of the reaction by which it fixes $CO_2$ suggests that evolution has made the best of a bad job.**

Rubisco is the enzyme in photosynthesis that is responsible for the conversion of inorganic carbon, as $CO_2$, into organic compounds. The demanding initial catalytic step (or carboxylase reaction[1]) precedes the photosynthetic reduction of reaction products using the energy trapped from sunlight. The acronym Rubisco actually stands for ribulose bisphosphate carboxylase-oxygenase, because the enzyme also has a tendency to confuse $O_2$ for $CO_2$ as its substrate. Rubisco has the reputation of being slow and inefficient, but it is one of life's big successes: globally there is an estimated 5–10 kg Rubisco for every person on Earth, and each year it reacts with 15% of the total pool of atmospheric $CO_2$.

## Active site

Work to 'improve' Rubisco and so increase crop productivity has usually foundered on the catalytic active site, which is highly conserved in different forms of the enzyme and has generally proved to be intractable to genetic manipulation. But a fresh angle on such prospects comes from Tcherkez and colleagues[2], writing in the *Proceedings of the National Academy of Sciences*. They propose an explanation for the reaction mechanism that accounts for the selection of $CO_2$ in preference to $O_2$. The systematic evolution of enzyme kinetic properties seems to have occurred in Rubisco from different organisms, suggesting that Rubisco is well adapted to substrate availability in contrasting habitats.

It is curious that Rubisco should fix $CO_2$ at all, as there is 25 times more $O_2$ than $CO_2$ in solution at 25 °C, and a 500-fold difference between them in gaseous form. Yet only 25% of reactions are oxygenase events at this temperature, and carbon intermediates 'lost' to the carbon fixation reactions by oxygenase action are metabolized and partly recovered by the so-called photorespiratory pathway. Catalysis begins with activation of Rubisco by the enzyme Rubisco activase[1,3], when first $CO_2$ and then a magnesium ion bind to the active site. The substrate, ribulose bisphosphate, then reacts with these to form an enediol intermediate, which engages with either another $CO_2$ or an $O_2$ molecule, either of which must diffuse down a solvent channel to reach the active site. Tcherkez and colleagues' achievement[2] is to have produced an explanatory mechanism for the trade-off usually observed between the specificity factor (that is, a ratio indicating selectivity for $CO_2$ over $O_2$, which ranges between 20 and 280; refs 1, 3) and $k_{cat}$ (the rate of enzyme turnover, which varies between two and eight catalytic events per second).

## Mutagenesis

Work leading up to this proposed mechanism[4] involved site-directed mutagenesis in tobacco, which had the aim of destabilizing the enzyme active site and altering the reactivity of the enediol intermediate. Changing the binding of an amino-acid residue that encourages the addition of both $CO_2$ and $O_2$ dramatically increased the rate of oxygenase activity.

Such observations[3,4] provided the key to the idea[2] that in the active site the enediol must be contorted to allow $CO_2$ to attack more readily despite the availability of $O_2$ molecules. The more the enediol mimics the carboxylate end-product, Tcherkez *et al.*[2] conclude, the more difficult it is for the enzyme to free the intermediate from the active site when the reaction is completed. When the specificity factor and selectivity for $CO_2$ are high, the impact on associated kinetic properties is greatest: $k_{cat}$ becomes slower.

So, rather than being inefficient, Rubisco has become highly tuned to match substrate availability. Several other correlates are also explained by this relationship. For instance, Rubisco discriminates more against $^{13}C$ than against $^{12}C$, the two naturally occurring stable isotopes in $CO_2$. But when the specificity factor is high, the $^{13}C$ reaction intermediate binds more tightly, and so carbon isotope discrimination is higher (that is, less $^{13}C$ is incorporated); in consequence, the carbon-isotope signals used to reconstruct past climates should perhaps now be re-examined. In contrast, higher ambient temperatures (30–40 °C) reduce the stability of the enediol, and Rubisco oxygenase activity and photorespiration rate increase.

These insights[2] into the mechanism of Rubisco catalysis are timely, because progress is being made in identifying the evolutionary origins of a wider range (forms I to IV) of Rubisco-like proteins[5]. The least effective of these forms have evolved, and now reside, in microorganisms in anaerobic sediments where catalysis does not have to compete with oxygen[1]. One bacterium can express all three catalytically active forms (I, II and III), and switches between them depending on environmental conditions[6]. The evolutionary