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# THE MORPHO-FUNCTIONAL ORGANIZATION OF SECRETORY TRAFFIC. HERE TODAY, WHERE TOMORROW?

ALBERTO LUINI[1] ALEXANDER A. MIRONOV[1],
MARIA ANTONIETTA DE MATTEIS[1] AND GARETH GRIFFITHS[2]
1Department Cell Biology and Oncology, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro (Chieti) Italy. 2EMBL Heidelberg, ALMF/Cell Biology/Biophysics Program, Meyerhofstr. 1, 69117 Heidelberg, Germany.

*SUMMARY*

*ORGANIZATION OF THE SECRETORY TRAFFIC*

*The morpho-functional principles of secretory traffic are still unclear, in stark contrast with our advanced knowledge of the underlying molecular machineries. Recently, the conceptual and technological hindrances that have delayed progress in this area have been disappearing, and new glimpses of the organization of traffic in intact cells are being revealed by a cluster of powerful morphological techniques. Here, we discuss the new advances in the light of the three main possible traffic principles: by anterograde vesicles, progression/maturation, and flow via continuities.*

Understanding how eukaryotic cells transport newly synthesized proteins from the endoplasmic reticulum (ER) to their particular cellular destinations has been a central goal of cell biology since the beginning of this discipline several decades ago. Progress throughout this period has been uneven, however, and the traffic field has gone through various different stages and shifts of focus[1]. One of the notable changes that has occurred over the last few years in the way we view intracellular traffic has been brought about by a re-examination, carried out in several laboratories, of the morpho-

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functional organization of the transport pathways *in vivo*. The key feature has been the use of a cluster of powerful morphological techniques to address in vivo long neglected but important aspects of the physiology of traffic. These are beginning to complement the long-standing success of cell free assays in identifying the molecular machinery involved in these processes. Here, we would like to summarize and discuss the conclusions, as well as the new questions, that have emerged from these studies.

*Origin of the gap between molecular and morpho-functional studies*

To put the new developments into perspective, it is useful to briefly summarize the origin of the persistent uncertainties that for decades have surrounded not just the structure and dynamics of the secretory membranes, but even the basic principles of transport. This is in striking contrast to the success at the molecular level, and has led to a significant conceptual gap between the two areas. The reasons for this situation are, in our view, both 'historical' and technological. One historical factor was the sheer elegance and simplicity of the vesicular model proposed by Palade decades ago and supported later by molecular studies[2-5]. This proposed that all the biosynthetic traffic is mediated by vesicles that bud from one stable compartment and fuse with another, with anterograde vesicles balanced by retrograde vesicles. This model, which became more or less accepted as dogma by a majority of specialists, came to dominate thinking in the 1980's and 90's, and the increasing information from the *in vitro* systems during this period were, for the most part, made to fit into the vesicular model. As a result, there was a decrease of interest in alternative traffic schemes. Another important factor was that as more molecular components were identified over the past 20 years, less emphasis was given to the morpho-functional aspects of intracellular traffic, and especially to the key question (with respect to the vesicular model): can it be shown directly in intact cells that cargo molecules in a given compartment A enter 'small' vesicles that then deliver this cargo to compartment B? Finally, and more importantly, this and other critical morpho-func-

tional questions proved to be technically extremely difficult to address. Indeed, until recently, they were beyond available technologies. To visualise traffic from, say, the ER boundary to the Golgi complex, one needed techniques to follow the process in a living cell. However, the only approach available for this purpose, light microscopy (LM), can only see a blurred blob in this region that has long been shown by electron microscopy (EM) to be an ultrastructural membrane jungle. In addition, the ultrastructural approaches themselves have had serious limitations. For instance, the specimen must be arrested by fixatives or cryo-immobilisation. Also, methods for the 3D reconstruction of intracellular membranes with a resolution sufficient to address the crucial question of connectivity between adjacent membrane compartments have emerged only recently.

*Novel microscopy approaches and their role in the recent advances*

It is only in the last few years that the traffic field has shifted focus again towards understanding the detailed organization of intracellular transport *in vivo*. This has been propelled by the development of new technologies and the growing awareness of the limitations of the vesicular model[6-10]. These novel microscopy methods have been a crucial factor in the field of biosynthetic traffic, and include a) green fluorescent protein (GFP)-based video LM for analysis of *in vivo* dynamic events[11], aided by developments such as fluorescence resonance energy transfer to follow protein-protein interactions *in vivo*, and improvements in resolution (see[12] for a recent review); and b) electron tomography, which has proven especially powerful for the detailed and faithful 3D reconstruction of small intracellular structures, and particularly of inter-compartment connections, when coupled to cryofixation[13-16]. These techniques have been complemented by useful ancillary approaches, such as correlative video light EM[17] which provides an interface between organelle dynamics and ultrastructure[18,19]; and have been aided by the development of a few new specialized synchronizable cellular systems which allow the transport of different cargoes to be fol-

lowed in time (see Figure 1)[20,21]. These and other approaches[22, 23] have provided evidence that anterograde vesicular traffic is neither the sole nor the main mode of transport, and they are opening the way to a new description of the organization of transport in intact cells.

In the following, we will discuss morpho-functional aspects of the various segments of the secretory pathway, with a focus on the principles of operation of traffic *in vivo*, and with the aim of emphasising not only the emerging concepts, but also the large knowledge gaps that the novel microscopy approaches are beginning to fill. In particular, we discuss the two standard models, bi-directional vesicular transport and compartment maturation (which so far have enjoyed the wider consensus), together with the third principle, ie. traffic via continuities[24,25]. We will analyse these schemes comparatively, *vis-a-vis* the available data, and point out their present limitations. The three traffic principles are schematized in Fig. 2.

*ER-to-Golgi transport*

The organization of the ER-Golgi transport through a very complex membrane system, often referred to as intermediate compartment, is still unclear even in its basic plan. We know that ER-Golgi carriers appear at specialized ER exit sites marked by the presence of proteins of the COPII complex[26-28] and by a tubular specialization of the ER membrane[29-31]. We also know the structure of at least one type of transport intermediate operating between these two stations in mammalian cells - a large pleiomorphic membranous container with saccular and tubular components[31-34]. However, we do not have conclusive evidence regarding how these intermediates form from the ER and how they mediate transport into the Golgi complex. It has also been proposed that the whole intermediate compartment might be a continuous system extending from the ER to the *cis*-Golgi[35], or that it might be discontinuous but linked by transient continuities (eg. by a kiss-and-run mechanism).

Two main ways (which are not mutually exclusive) have been proposed to explain the formation of ER-Golgi carriers. According

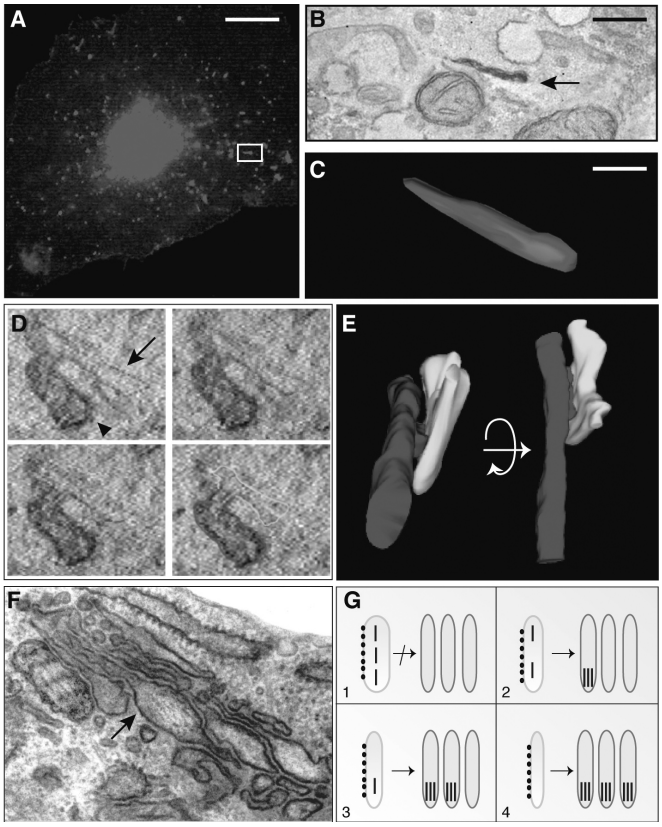


Fig. 1 - Modern approaches in morpho-functional studies of the secretory pathway. (A-C) Correlative video EM of a VSVG-containing post-Golgi carrier. The carrier of interest (white box) is followed *in vivo* by GFP-based video-microscopy (A), fixed at a time chosen by the observer, and immunolabeled (in this case by the immuno-HRP technique) (B, arrow). Its 3D ultrastructure is then reconstructed from serial thin (60 nm) sections (C). Bars: 7 m (A), 0.75 m (B) and 0.29 m (C). (D, E) Electron tomography of a forming ER-Golgi carrier. The carrier is followed as described in (A), fixed, and cut in thick (200 nm) sections, each of which is analysed by electron tomography. Four virtual serial sections (5 nm thick) of the carrier are shown in (D), and the complete 3D model, seen from two different angles, in (E). The 3D resolution is several-fold higher that in traditional reconstructions (eg. that in C). The ER (arrow) is dark grey and the forming carrier (arrowhead) is light grey. (F, G) Synchronization of transport of large supramolecular cargo. (F) shows a Golgi complex of a chick embryo fibroblast exhibiting a procollagen aggregate (arrow) in each cisterna and (G) a PC synchronization scheme [20]. PC molecules (black lines) are arrested in the ER by blocking proline hydroxylation. As a result, the secretory pathway is emptied of PC (G1). The block is then removed, and the PC aggregates can be observed to progress through the Golgi stack synchronously (G2-4).

to one, cargo protein is concentrated within small round COPII coated vesicles that physically separate from the ends of the ER. These subsequently uncoat and fuse with each other to form the large carrier destined for the Golgi (a variation of this scheme envisions that some soluble proteins are concentrated after exit from the ER by a COPI-dependent mechanism; see[36]). This model is compatible with most of the *in vitro* molecular evidence[37-39] (but see

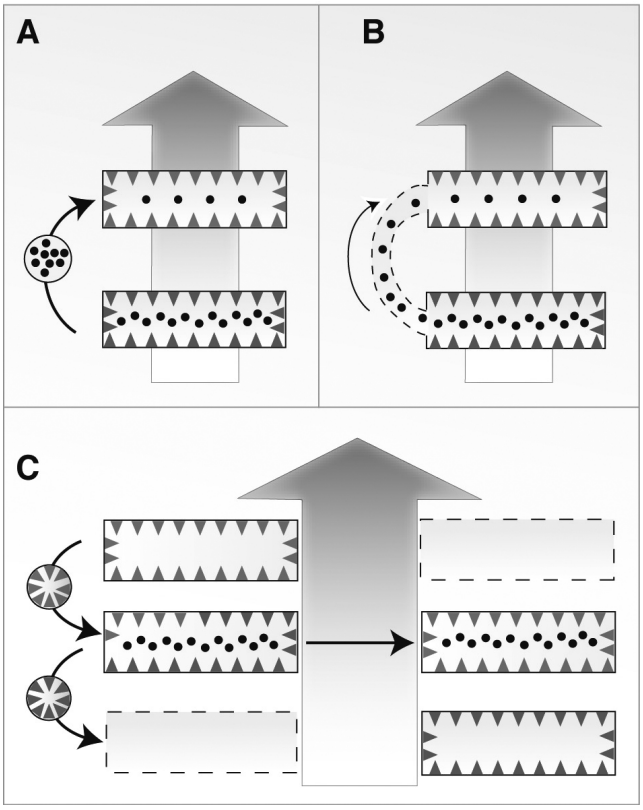


Fig. 2 - The traffic principles. (A) Anterograde vesicular traffic. Compartments are stable. Cargo moves via vesicles from proximal to distal compartments. (B) Flow via continuities. Cargo moves from proximal to distal compartments via tubular continuities. (C) Progression-maturation. The components of distal compartments move backward into the next proximal element. As a result, the cargo appears to shift from a proximal into a distal compartment.

also[40] and with some, but not all, of the ultrastructural observations in mammalian cells. It has one major difficulty, however. It does not easily account for the export from the ER of molecular complexes larger than COPII vesicles, such as procollagen (PC), lipoprotein particles or lipid droplets[41,42] (unless one proposes that transport vesicles might be 'elastic' enough to accommodate large cargoes; see[43]). The export of these cargoes is instead easily explained by the second model, according to which ER-Golgi carriers emerge by *en bloc* protrusion of large portions of specialized ER exit surface[44] (the two models are schematized in Fig. 3).

Both schemes enjoy some experimental support. The *en bloc* formation scheme is favored by *in vivo* morphological evidence indicating that it operates in the exit of PC from the ER[45]; it is therefore likely to be correct at least for this macromolecular cargo class. As for the vesicular model, the formation of COPII vesicles and their ability to deliver cargo to acceptor compartments has been documented by *in vitro* experiments (see[46]), and in yeast[47]. On this basis, the idea that free COPII vesicles form from the ER also *in vivo* in mammalian cells has been widely accepted. However, in these cells, direct evidence for this model has been so far technically difficult to obtain. It is therefore possible that the *en bloc* formation scheme might apply not only to exit of large macromolecules, but also to small diffusable cargoes, at least in mammals (while the vesicular exit mechanism might prevail in yeast). Adequate *in vivo* approaches to tackle the question are now available, and it should be possible to discriminate between the two schemes, for instance by examining the ultrastructure of these intermediates during their formation by the previously mentioned methods, and/or by determining the effect of fusion blockers on the ultrastructure of the forming intermediates, similar to a previously proposed approach[48] (if they result from the fusion of small vesicles, they should appear as vesicular clusters when fusion is inhibited). We have recently undertaken an extensive analysis of cargo export from the ER which has indicated that COPII provides coats to subdomains of large cargo-containing membrane protrusions from the ER, and that free cargo-containing

vesicles are very scarce (our unpublished data). How would one fit this view with the extensive and elegant series of data from Schekman and his collaborators? One possibility, as pointed out by others, is that yeast might operate differently from mammalian cells. If not, we suggest that the COPII-containing membranes isolated from yeast may have other membrane domains attached to them that would represent the equivalent of the forming pleiomorphic carriers we see in higher eukaryotes.

Clearly, whichever model turns out to apply in living cells, it will

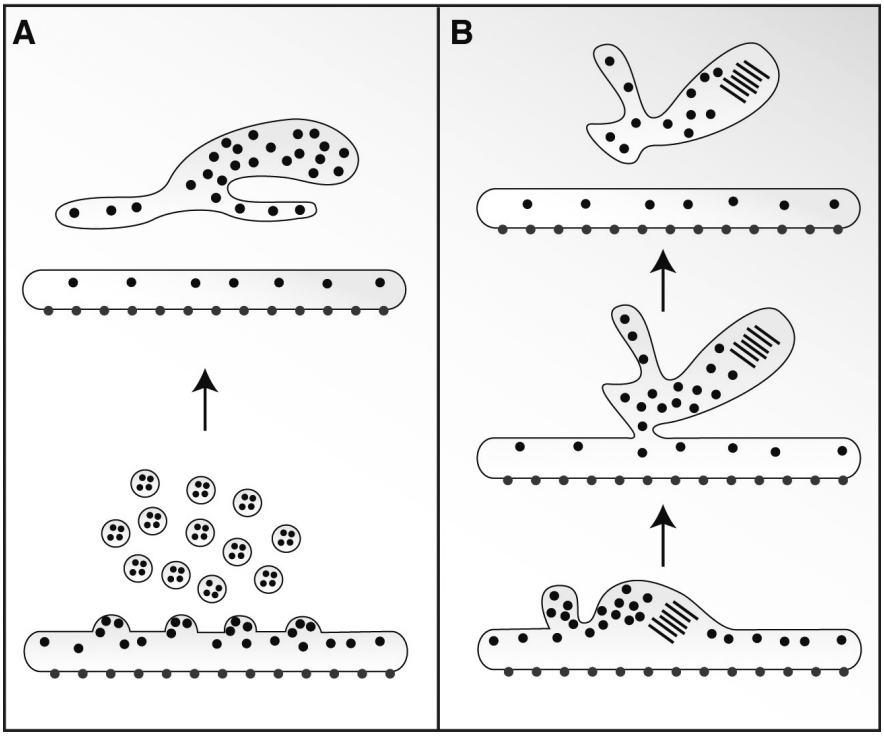


Fig. 3 - Formation of ER-Golgi carriers. (A) Budding and fusion of vesicles. Cargo (black dots) is concentrated in ER buds. This is followed by vesicle formation and fusion into a large pleiomorphic carrier. (B) *En-bloc* protrusion from ER membranes. Note that only the model in (B) explains the export of PC (black lines). The possible schemes of exit from the TGN are likely to be similar, in spite of mechanistic differences (see text).

have to accommodate the indisputable fact that a central role in ER export is played by the COPII machinery for both small and large supramolecular cargo[49]. The model will therefore need to reflect the proven properties of this complex, namely, its abilities to induce membrane bending[50] and also to sort and concentrate cargo [51]) as well as traffic machinery components such as the SNARE and the rab[52] proteins involved in docking and fusion.

After formation, and before moving away from the ER, the available data suggest that newly formed carriers must bind the COPI coat to 'mature' and proceed towards the Golgi[53]. Also for COPI, it is widely assumed that free vesicles form; again, definitive evidence in cells is still lacking. The centripetal movement of carriers is mediated by microtubules and is usually implied to involve physical detachment of the container from the parental ER membranes. It is also conceivable, however, that, at least in some cases, cargo flows along preexisting tubular structures. Such a 'bolus' traffic mode[54] is mechanistically difficult to envisage, but it has been described for the endocytic pathway[55] and should not be completely ruled out at this stage also in the biosynthetic route. The new LM and EM technology might allow the verification of this intriguing idea.

Finally, when the ER-Golgi carriers reach the Golgi area they must discharge their cargo in the Golgi complex. Here, again, there are a few different models between which we are presently unable to distinguish. In brief, one model envisions that cargo-loaded vesicles detach from the ER-Golgi shuttle and fuse with the *cis* cisterna. An alternative is the progression/maturation scheme, by which ER-Golgi carriers homotypically fuse with each other into a new cisternal structure at the *cis* pole. Interestingly, consistent with this possibility, 'mature' carriers on the way to the Golgi complex have been reported to interconnect extensively, in living cells, suggesting that they can fuse before reaching the central Golgi area[56]. Yet another scheme posits that the ER-Golgi carriers fuse *en bloc* with the *cis*-Golgi cisterna. Thus, little is clear about the organization of this crucial step. Once more, the solution to this problem will probably have to rely on the use of precisely synchronized secretory sys-

tems and on resolving the dynamics and the fine structure of the carriers during their arrival at the *cis*-Golgi. Another useful approach will probably be to explore the morphological effects of inhibiting specific SNARE[57-59] or tethering[60] proteins implicated in this step.

*Traffic through the Golgi stack*

A serious complication in understanding how secreted material traverses the Golgi stack is the fact that the organisation of the Golgi complex is still poorly understood; in this respect, the word "complex" is an apt one! Although it is likely that the use of EM tomography will eventually solve the structure of this organelle, this will not be a trivial task. Moreover, a reconstruction of the structure *per se* will not solve the problem; ultimately, the morpho-functional approach demands that defined cargo at precise and sequential stages of transport be mapped at the ultrastructural level. Such knowledge of the organization of the Golgi will determine how transport through this complex proceeds. A critical issue, in particular, is whether the Golgi consists of several stably separate compartments or whether these compartments can establish continuities, even transiently.

In recent *in vivo* experiments, an advantage has been the use of a synchronizable secretory system that is able to transport not only small diffusable molecules (eg, the widely used traffic marker VSVG viral protein;[61]) but also supramolecular complexes (PC aggregates) much too large to enter Golgi 50nm COPI vesicles or to diffuse freely along membranous continuities. Using this system, both PC aggregates and small VSVG cargo have been shown to move at the same rate through the Golgi complex, without entering Golgi vesicles, and while remaining in continuity with the lumen of cisternae. In addition, both PC (as expected) and VSVG were found to traverse the Golgi without diffusing laterally long distances along the ribbon.

These surprising results put crucial constraints on the way we should think about intra-Golgi traffic. They are inconsistent with the anterograde vesicular model (at least for VSVG and PC; of

course, it cannot be excluded that other cargo proteins or lipids might use anterograde vesicles; see[62]) and with published versions of the traffic model by diffusion via continuities along the Golgi. At first glance, these data are instead compatible with the progression/maturation model. Although some degree of consensus has been recently forming behind this idea, at this stage we think that this conclusion is premature, and other possibilities should be left open. Our reasons for caution are as follows. A key prediction of the maturation model is that Golgi enzymes concentrate in COPI-dependent vesicles, within which they move backwards through the stack[63, 64]. While some evidence in support of this possibility has been produced recently, ie: a) vesicle-like Golgi membranes containing a high concentration of Golgi residents have been isolated *in vitro*[65]; and b) peri-Golgi round profiles (possibly sections of vesicles) have been shown to contain the Golgi enzyme mannosidase II (ManII) *in vivo*, these observations do not seem conclusive because: 1) the concentrations of ManII found in vesicular profiles are not significantly higher than those in cisternae (at variance with the prediction of quantitative maturation models; 2) other authors do not confirm this observation[66]; 3) the ManII-containing profiles seen *in vivo* have not yet been shown to represent vesicles, rather than cross sections of tangential tubules (or perforated cisternal rims); 4) only ManII has so far been found in 60 nm round profiles in significant amounts *in vivo*, whereas other enzymes and Golgi resident proteins should be studied *in vivo* before any general conclusion can be drawn; and 5) the *in vitro* isolated Golgi-enzyme-containing vesicular membranes have yet to be shown to be able to fuse with Golgi cisternae (a prediction of the maturation model;[67]).

Finally, the key *in vivo* evidence so far presented for maturation, ie. the permanence of cargo within the lumen of cisternae during traffic, is compatible, in mammalian cells, with alternative models based on gated continuities between heterotypic cisternae. While technically difficult to visualize because of the 3D complexity of the Golgi (however, see[68,69]), such continuities might transiently form in the Golgi ribbon in the tubular-reticular zone interconnect-

ing adjacent stacks, whose very complex morphology is still incompletely understood. An objection to this might be that even scattered Golgi stacks, such as those found in plants and in microtubule-deprived cells[70,71] (where inter-stack connections obviously do not exist), execute transport as efficiently as Golgi ribbons. However, it is possible that the cisternae of these separate stacks establish continuities between themselves, which would be functionally equivalent to those found between adjacent stacks in Golgi ribbons[72]. The presence of such connections must still be verified, but preliminary evidence indicates that they do exist[72].

Two of these continuity-based models are presented in Fig. 4. In the first (Fig. 4B), Golgi enzymes flow backwards through continuities across heterotypic cisternae while the cargo does not move. This would be essentially a maturation scheme via gated continuities, rather than via vesicles. In the second (Fig. 4, panels C1-2), it is the cargo that moves anterogradely via gated connections, while cisternae remain stable (more details on these models are given in Fig. 4 and its legend). Experimentally, gated-continuity models could be discriminated from vesicle-based maturation by determining the precise 3D location and concentration of Golgi enzymes during a controlled wave of traffic through the Golgi. Of course, these models generate mechanistic questions: for instance, one would have to explain how directionality of flow and segregation between cargo and enzymes (a crucial event in these schemes; see legend to Figure 4), and between different Golgi compartments or sub-domains, would be achieved. Moreover, they cannot be directly applied to yeast, where the anatomy of the Golgi is very different[73]. Also, the anterograde cargo flow scheme (Fig. 4, panels C1-2) is difficult to apply to some scale-secreting algae[74] where scales are so large as to fill entire cisternae. Thus, the overall organization of intra-Golgi traffic in such phylogenetically distant cell types might be different. However, because gated continuities are not mechanistically unfeasible and are compatible with existing data in mammals, we view these models as viable and worthy of experimental verification.

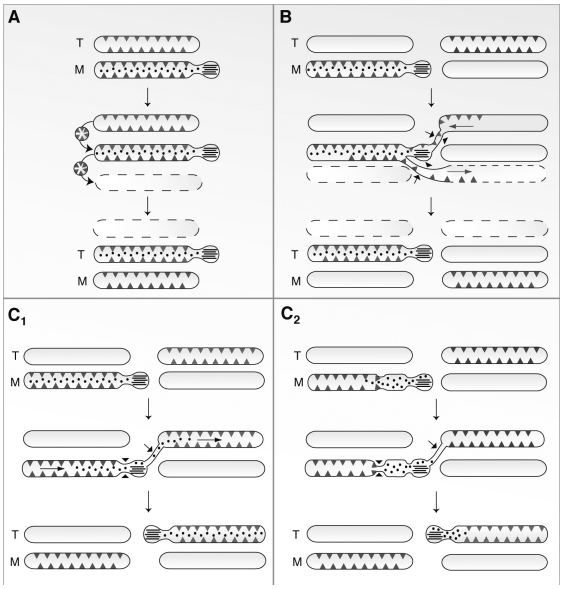


Fig. 4 - Models of intra-Golgi traffic. (A) Cisternal maturation by retrograde COPI vesicles. Golgi enzymes hop backward from a *trans* (T) to a medial (M) cisterna within COPI vesicles. The concentration of enzymes (triangles) is higher in vesicles than in cisternae. Both diffusable and large supramolecular cargo (black dots and lines, respectively) remain in the cisternal lumen. In this model, the segregation and concentration of enzymes from cargo is coupled with vesicle formation. (B, C) Traffic via gated continuities from a medial to a *trans* cisterna. For simplicity, only the cisternae involved in the traffic event are shown to contain cargo and enzymes. Arrows indicate fusion, arrowheads, fission. (B) Retrograde flow of Golgi enzymes. A transient connection is established between M and T cisternae, through which *trans* enzymes, but not cargo, move backward into the medial element. The connection is then interrupted by fission. This model implies lateral segregation between cargo and enzymes and retrograde movement of the latter. In a Golgi ribbon (schematized here as two stacks) connections can occur between adjacent stacks. In the separate stacks of plants or nocodazole-treated cells, inter-cisternal connections might occur within the same stack. (C). Anterograde movement of cargo. (C1) A connection is established, through which cargo, but not enzymes, moves unidirectionally from a medial into a *trans* cisterna. This is followed by fission. The underlying mechanisms are analogous to those posited in (B): segregation of cargo from medial enzymes followed by movement into the *trans* cisterna and by mixing with *trans* enzymes. Traffic of large cargo (PC) might happen by fusion of a medial PC-containing distension with the *trans* cisterna, followed by fission of the distension from the donor element at a site proximal to the distension itself (arrows), completing the transfer. (C2) Similar to (C1), except that not only supramolecular, but also small diffusable cargo remain in domains partially segregated from enzymes at all times during transport, ie. they behave like PC. In the (C) schemes, to account for the similar transport rates of PC and VSVG, and for the lack of VSVG diffusion throughout the stack during transport [16], the transfer of soluble cargo must be complete before fission occurs. Under all these schemes, the cargo remains in continuity with the lumen of cisternae, as required by the experimental observations.

*Intra-Golgi transport and the shape of the stack: a possible role of COPI vesicles*

How is the ordered, yet dynamic, structure of the Golgi maintained, and what role might this have in intra-Golgi traffic? We propose that the relationship between structure and function of the stack might be mediated, in part, by the COPI vesicles. While, as noted above, COPI vesicles might act as retrograde carriers of Golgi enzymes (within the maturation scheme) and, possibly, as anterograde carriers of specialized proteins, the COPI complex[75,76] has also been credited with a predominant role in controling the morphology of the Golgi stacks. However, the precise mechanism of such control is not understood. There are at least two aspects to the question of the Golgi shape. One concerns the molecules providing cohesion between Golgi membranes. These have been studied extensively, and the group of proteins collectively termed the Golgi matrix, which include the GRASPs and the golgins, have been shown to participate in the stacking of cisternae, both *in vivo* and *in vitro*[77-79]. However, there is a second crucial aspect which has been given less attention: the control of the geometry of the Golgi membranes. We propose that this is the mechanism in which COPI vesicles play a role.

Golgi elements at steady state can be classified into cisternae, tubules and vesicles. Transformations between these geometries can take place very rapidly (within seconds to minutes), indicating that they exist in a dynamic equilibrium. A well-known example is the effect of brefeldin A, a fungal toxin which causes a rapid and reversible tubulation of the Golgi cisternae[80,81]; fast shape and curvature changes also occur during normal traffic. Notably, changes in curvature are necessarily coupled to large modifications in transmembrane surface asymmetry[82-84], for which energy is necessary, as well as specific mechanisms. While such mechanisms might be multiple[85, 86], we propose that a major factor for the rapid control of Golgi transbilayer asymmetry might be the formation itself of highly curved COPI vesicles from cisternal membrane. This is because the formation of vesicles 'extracts' membrane asymmetry from the

cisternae. This effect, in turn, tends to maintain the flat geometry of the cisternae themselves. Conversely, when coatomer is inactivated (eg. in the presence of brefeldin A), vesicles can no longer form, but they can still fuse with cisternae. This will inject asymmetric vesicular membrane into the cisternae, whose curvature will increase as a result, and change the cisternae into tubules. In other words, COPI vesicles could function as 'reservoirs' of membrane asymmetry (curvature) available for rapid local shape changes of Golgi cisternae, for transport or for other needs. This proposal can in principle be tested by blocking vesicle formation and/or fusion, and measuring the overall degree of transmembrane asymmetry of the Golgi complex in the two (tubulated versus stacked) configurations using available morphometric techniques.

Closely connected with this idea is the question as to whether these shape phenomena could be involved in traffic. In this regard, it might be significant that not only the coatomer but also the golgins interact with the GTPases known to have a role in traffic[87,88] and also with the putative cargo-receptors of the p24 family[89]. In addition, the golgins might interact with Golgi enzymes[90]. Thus, golgins might participate in Golgi dynamics, and possibly in transport, by mediating segregation/desegregation events between cargo- and enzyme-containing domains. As for COPI, it could control the extension of transient tubules (a localized burst of vesicle fusion might induce local tubulation), establishing continuities between adjacent cisternae. Coordinated tubulation and segregation events (under the gated continuities models) have the potential to regulate traffic through the Golgi (see Fig. 4 and its legend).

*Exit from the Golgi complex*

Once the cargo reaches the *trans*-Golgi network (TGN), it must leave for one of several intermediate or final destinations. The TGN is a complex organelle consisting of a cisternal saccular portion connected with a convoluted tubule system[91-93]. The principal transport functions of the TGN are cargo sorting, export and recycling. Sorting seems to rely on cytosolic protein adaptors (AP1, AP3, AP4

and the GGAs), each of which probably specifies a cellular destination by interacting with sorting signals on the cytosolic tails of cargo molecules (reviewed in[94]). But how this process is coupled to the formation of traffic intermediates and how such intermediates are generated is again not clear. Post-Golgi carriers directed to the plasma membrane are pleiomorphic tubular saccular structures, often (but not always) very large (eg. a few microns in length, almost as large as a Golgi cisterna), and can be seen to be pulled out of the Golgi mass as long membranous tubules, a process facilitated by microtubule-based motors. This morphology brings back a question already discussed for ER-Golgi carriers. Are these intermediates generated by budding of small vesicles which later fuse with each other, or do they form by protruding *en bloc* from the TGN (similar to the scheme in Figure 3B for exit from the ER)? Although a conclusive characterization of the formation process has not yet been done, there are hints that the latter mechanism applies. For instance, in cells secreting both PC and VSVG, the two cargoes exit the Golgi in the same containers (and, obviously, PC aggregates do not fit into small vesicles); when a tubular carrier growing out of the Golgi detaches from the microtubule along which it is being pulled, it appears to retract elastically, suggesting that its base is still embedded in the TGN[95]; finally, in cells transfected with a dominant negative protein kinase D mutant proposed to block fission, carrier tubules are extruded from the Golgi but are unable to detach from the Golgi mass, again suggesting that they are linked to it[96]. An answer, once again, might come from the use of the morphological approaches described in the case of ER-Golgi carriers.

It is worth noting that while the morphology of the post-Golgi carriers is reminiscent of the ER-Golgi intermediates, the mechanisms of formation of these two structures may not be similar. While the latter depends completely on the COPII machinery, the requirement for adaptor proteins for post-Golgi carriers is not absolute[97-99]. Moreover, interestingly, their formation from the Golgi appears to require a 'lipid machinery'. Known lipid components include the phosphoinositides (reviewed in[100]), phosphatidic

acid[101-104] and diacylglycerol[105], and some of the involved lipid-handling proteins have been identified[106-109]. This 'lipid machinery', however, is sure to be quite dynamic and complex, and we suspect that we are now seeing only the tip of the iceberg. We consider it likely that some lipids act not only as docking sites for 'fission' proteins, but also as modifiers of the geometry of the bilayer through their non-cylindrical shape, thus somehow relieving the need for a protein coat, and directly resulting in local membrane bending, tubulation, and, later, fission.

*In vivo role of the traffic molecules*

This tour of the secretory pathway leaves us not only with glimpses of a new view of intracellular transport, but also with the awareness of large knowledge gaps. The task of completing the picture, however, is no longer as daunting as it seemed up to a few years ago, because the technological and conceptual tools to resolve the issues are now available. It is crucially important to continue to document carefully both the ultrastructural and the dynamic organization of the biosynthetic pathway. This will require state-of-the-art LM and EM approaches. While expertise in LM is rapidly expanding, the critical bottle-neck will be the availability of technology and expertise in EM, as already discussed[110].

A separate, but closely interlinked goal will be to fit all the molecular information into the morpho-functional map of the biosynthetic pathway. While the body of knowledge so far generated on the composition and the elementary properties of the traffic machineries (eg. membrane bending, fusion, sorting, etc) is impressive, in most cases a large gap remains between these *in vitro* properties and the *in vivo* role of the molecules. Crucially, assigning *in vivo* functions will only be possible when a clearer understanding of the morpho-functional organization of the system is available. It is therefore desirable to increasingly integrate molecular studies with the morpho-functional approaches discussed here. However, to this end, another crucial tool needs to be developed. This is the ability to rapidly block the function of each molecular machine. This pos-

sibility is already available in yeast, where several temperature-sensitive mutants can be inactivated within minutes. In contrast, in mammalian cells the function of a protein can only be ablated by chronic treatments, which enormously complicates the phenotype (with the exception of microinjection, which has provided a partial solution in some cases). New efforts to create fast-acting tools are being made, for instance towards developing membrane-permeant drugs able to affect specific cellular machineries[111,112]. Nevertheless, simple and efficient technologies designed to deliver polypeptides such as dominant-negative mutants or specific protein domains, onto intracellular targets (see[113]) are still needed in order to use morpho-functional assays to examine the precise function of traffic molecules in the *in vivo* context.

Beyond the questions discussed above loom deeper problems. We would like to know, for instance, what controls the homeostasis of the traffic organelles both in terms of structure and dynamics, and how they are regulated in concert with other cellular functions. In this regard, a major role is starting to be played by the postgenomic methods[114-116], leading towards a complete repertoire of the traffic molecules, and maps of their physical and functional interactions. We foresee that the integration between the genomic methods, the new morphological techniques, and the still crucial reductionist approach, will provide a massive impulse in the next decade towards a faithful representation of the *in vivo* physiology of secretory traffic.

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Correspondence should be addressed to:
Alberto Luini, Department Cell Biology and Oncology, Consorzio, Mario Negri Sud, 66030 Santa Maria Imbaro (Chieti) Italy, Tel. 39-0872-570355; Fax 39-0872-570412 e-mail: luini@dcbo.negrisud.it