Constitutive transport between the *trans*-Golgi network and the plasma membrane according to the maturation model. A hypothesis

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Abstract Here we examine the application of the cisternal/carrier maturation model to describe transport of cargo proteins from the Golgi apparatus to the plasma membrane. Interpretation of the available evidence in the light of carrier maturation suggests that the transport intermediates between these stations are large pleiomorphic carriers formed by maturation of the *trans*-Golgi compartment, rather than vesicles, as would be postulated by the vesicular shuttle model. Mature carriers move along microtubules towards the plasma membrane via a microtubule/(kinesin)-based motor system. The maturation and vesicular transport models are compared in terms of consistency with the available literature.

Key words: Golgi-plasmalemma transport; Carrier maturation; Lateral diffusion; Cisterna maturation

1. Introduction

According to the vesicular shuttle model of intracellular traffic, secretory proteins move through the biosynthetic pathway from each compartment to the next in discrete membrane-bound vesicles [1]. Over the last 15 years this scheme has incorporated a large number of experimental findings and become so universal a paradigm of intracellular traffic that it has been extended even to transport steps for which putative carrier vesicles have not yet been clearly identified, such as that from the *trans*-Golgi network (TGN) to the plasma membrane (PM). Recently, however, both new observations and current reappraisals of earlier data have brought to the fore traffic models based on different principles [2].

The so-called cisternal progression maturation model, which is attracting much interest, explains intra-Golgi traffic by the gradual maturation of Golgi cisternae [2,3]. A key feature of cisternal maturation is that it accommodates the well-known fact that a number of important proteins are transported and secreted as large aggregates [4,5], an observation which, by contrast, the vesicular shuttle scheme does not easily fit. According to the maturation scheme, each Golgi compartment matures by losing its enzymes, possibly via retrograde vesicular transport, and acquiring those of the distal compartment. A medial cisterna, for instance, becomes a new *trans*-Golgi (TG)/TGN compartment by acquiring TG/TGN enzymes and losing its own enzymes to the *cis* compartment. As a result, new cisternae must be constantly formed at the *cis* side and the TG/TGN compartments consumed by secretion (see [2] for a detailed description of the model). Here, we analyze the consequences of the cisternal maturation mechanism for TGN-PM traffic by consumption of the TGN and compare this with the vesicular shuttle scheme in the light of the available evidence at this stage of transport.

We propose that large supramolecular aggregates (SA) remain in carriers formed by maturation of cisternae/tubules, through selective depletion of the TG/TGN cisternae/tubules via retrograde transport to the preceding Golgi compartment of most of its defining components. In the light of the existing evidence, this idea appears sound and deserves to be tested. We also propose that this mechanism might be general and equally applicable to small diffusible cargoes. It must be specified, however, that the object of our analysis is constitutive transport between the TGN and the PM. Regulated secretion might be different [6] and thus will not be discussed in this context.

2. Maturation versus vesicular traffic

2.1. In vivo evidence

As briefly noted above, strong support for the maturation model of constitutive TGN-PM transport stems from the same type of observations that have inspired the intra-Golgi cisterna maturation scheme [2]. In a number of diverse cell types, SA are found in the TG/TGN: alga scales [7,8], apolipoprotein E-containing particles in liver cells [9,10], lipid droplets in enterocytes after oil feeding [5], aggregates of procollagen in fibroblasts [4], eccentric electron-dense spherical bodies in epithelial cells of the seminal vesicle [11], asymmetric membrane thickening of the apical plasmalemma of uroepithelial cells [12], and casein submicelles in lactating mammary gland cells [13]. It is also worthy of note that in polarized cell systems some of these SA are transported towards the basolateral PM: lipid particles and droplets, procollagen aggregates. Others, spherical protein bodies and membrane thickening, are delivered to the apical plasma membrane. Their common feature is that they are constitutively transported to the PM but are simply too big [2] to be carried by the 60–100 nm vesicles proposed to be TGN-PM carriers in the vesicular model [6]. The size of these cargoes does not pose a problem for the maturation model which affirms that supramolecular aggregates are transported within specialized membrane envelopes that are depleted of TG/TGN enzymes by retrograde cycling (but retain components required for transport, docking and fusion).

In principle, there are ways to accommodate the transport of SA within the vesicular shuttle mechanism, or modified versions of it. It could be proposed, for instance, that coated vesicular intermediates of bigger size could serve as transport

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intermediates instead of small vesicles. An example of such a possibility has been reported at the plasma membrane level [14]. Another objection to the maturation model is that SA might disassemble into smaller subunits and be packaged into conventional transport vesicles. Although these possibilities cannot be ruled out, they do appear unlikely: SA-containing vacuoles located in the close vicinity of the TGN never exhibited visible coats (although sometimes clathrin-coated buds are visible on them) and, at least in the case of procollagen granules, their content is tightly packed, rendering the disassembly-reassembly mechanism implausible. Finally, many SA would be too big to fit into transport intermediates even if they did undergo disassembly. For instance, procollagen folds inside the endoplasmic reticulum (ER) into 300 nm long, very stable, rigid and almost inflexible triple-helical rods [15]; they cannot be packed into 60–100 nm vesicles considered to be TGN-PM carriers. On the other hand, 300 nm long coated tubules have never been observed near the *trans* side of the Golgi stack [4].

As for the transport of small diffusible molecules (as distinct from large aggregates), recent observations in living cells indicate that TGN-derived carriers are of 300–600 nm in diameter (much larger than 'classical' vesicles) and irregular in shape. They contain cargo (green fluorescent protein (GFP)-tagged chromogranin), are devoid of TGN and endosomal/lysosomal markers and migrate via rapid discontinuous movements with frequent reversals of direction and maximal velocities of 1 μm/s [16]. A similar approach using GFP-tagged vesicular stomatitis virus glycoprotein indicated that cargo-containing tubule-shaped structures detached directly from Golgi, in a microtubule (MT)-dependent manner [Hirschberg et al., Mol. Cell Biol. 8 (Suppl.), 194a]. Moreover, recent evidence obtained through a combination of high resolution laser scanning confocal microscopy and electron microscopy demonstrates that synaptic, plasma membrane and TGN proteins are all transported from the TGN to the apical (axonal) plasma membrane via tubulo-vesicular structures of various sizes [17]. All the same, these findings appear compatible with the idea that areas of the membrane-bound structure resulting from TGN maturation (by loss of TGN markers) become accessible to MT-based motors and are consumed by breakdown into large cargo-containing fragments before moving to the PM. The same results, however, could also be explained suggesting that the formation of small cargo-containing coated vesicles from the TGN is followed by their fusion into a large tubular-vesicular intermediate. Thus, although the vesicular model can accommodate the recent results from time-lapse imaging studies, these are more consistent with the maturation model. The vesicle-shuttle model remains inadequate to explain the observations showing TGN-PM transport of SA incompatible in size with conventional vesicular carriers.

The maturation model implies the existence of TG/TGN-derived retrograde vesicles (or other carriers) that exclude cargo and are enriched in resident proteins. The study of the characteristics of TGN-derived vesicles has thus the potential to distinguish between the two models. Indeed, TGN- and TG-derived clathrin-coated buds have been shown to contain Golgi-resident proteins [18] but to exclude cargo [19]. Furthermore, experiments with yeast mutants revealed that clathrin plays a direct role in the retention of Golgi-resident proteins by preventing their transport to the PM [20]. Finally, it has been demonstrated that coatomer, generally implicated in retrograde transport, is required for the formation of TGN-derived carriers containing cargo in vitro [21], suggesting that coatomer-coated transport intermediates containing Golgi enzymes might participate in carrier maturation. The recycling components of the TGN might first segregate within tubules (prior vesicular transport to the *trans* cisterna), perhaps in the same way in which recycling endosomal components are separated from fluid phase endocytic markers and shipped back to the PM [22]. Tubules might then be synchronously broken into the rows of vesicles observed with electron microscopic tomography [23].

2.2. In vitro evidence

Over the last 10 years, a number of reports have indicated that, under proper conditions, isolated TGN-derived carriers enriched in cargo, devoid of lysosomal/endosomal proteins and able to deliver cargo to the PM, can be formed in vitro. These carriers appear vesicular in nature [6]. This conclusion, however, suffers from the weaknesses arising from all extrapolations from the interpretation of in vitro data to physiological events. In a series of experiments designed to generate TGN-derived transport intermediates (TI), semi-intact cells or isolated Golgi membranes were incubated with cytosol in the presence of an ATP-regenerating system and GTP after preincubation at 20°C, a temperature known to block TGN-PM transport and induce the accumulation of cargo in the TGN. TI isolated under these conditions appear as small smooth membrane vesicles of irregular shape without a distinct coat and with diameters ranging from 50 to 200 nm [19] or 80 to 200 nm [24]. This irregularity is a very surprising feature for isolated coated vesicles, proposed to have a uniform size and a spherical shape [25], and in fact suggests that their mechanism of formation might not directly involve a protein coat. Thus, a possibility in line with the maturation process is that these TI result from the fragmentation of larger tubular carriers during the isolation procedure. It is in fact known that mechanical stress is deleterious for tubulated structures, both in vitro and in vivo, and usually leads to their breakdown into tubulo-vesicular elements [26]. These fragments would have round or oval profiles in thin sections and be rather irregular in shape and heterogeneous in size. Thus, experiments viewed as strong evidence for the vesicular model of TGN-PM constitutive transport in fact suffer from some over-interpretation.

In other studies, the vesicular nature of TGN-derived carriers was suggested by the formation of cargo-containing coated vesicles under conditions stimulating the irreversible activation of small G-proteins (by mutation or by non-hydrolyzable GTP analogs). It has been often pointed out however, that GTP analogs might produce non-physiological events. For instance, in permeabilized cells and in the presence of irreversibly activated Sar1p, the mostly tubular ER-derived TI acquire the appearance of lace-like coated structures connected with the ER [27]. A similar phenomenon was demonstrated with Golgi membranes when, in the presence of a non-hydrolyzable GTP analog, Golgi tubules were transformed into rows of coated vesicles connected by fibrous bridges after binding of COPI [28]. Considering that coatomer, clathrin and myosin II, the coat proteins proposed to act at the TGN, are all regulated by G-proteins [1], it is reasonable to expect that the generation of TI in the presence of activated G proteins

would be accompanied by the fragmentation of its cargo-enriched tubules into coated vesicles. Indeed, in the presence of non-hydrolyzable GTP analogs, TG-derived carriers appeared as coated vesicles [6,25]. Correspondingly, the vesicular appearance of TI in the presence of irreversibly activated G-proteins might be due to a non-physiological in vitro vesiculation of larger tubular carriers. In conclusion, there are uncertainties as to whether such evidence actually reflects the physiology of traffic in vivo.

3. The maturation model

Analysis of the literature clearly indicates that the vesicle-shuttle model is not the only possible mechanism compatible with data describing TGN-PM traffic and as yet cannot deal with the transport of SA. Here we present an alternative framework with similar, if not higher, explanatory power. The carrier maturation model implies that the trans cisternae connected [23] to the TGN undergo maturation by recycling of all resident TG/TGN enzymes through a coat-based (or other) mechanism analogous to retrograde coatomer (or clathrin or myosin II)-dependent vesicular transport. As a result, the TGN is transformed into cargo-containing carriers of tubulo-saccular appearance, depleted of resident proteins, and able to use a MT/kinesin-dependent motor system for their delivery to the PM. Connections with the TGN might be preserved during carrier movement, at least for some period, thus generating a route for lateral diffusion of lipids. Long processes emanating from the TGN have indeed been described in living cells [29], and labeled lipids have been reported to diffuse to the Golgi complex even in aldehyde-fixed cells, suggesting the existence of continuities between the Golgi and the PM [30].

4. Sorting of cargo: implications of the maturation model

The TGN is quite unique in that proteins are sorted from it to at least two to four different destinations depending on the cell type (basolateral or apical PM, lysosomes and secretory granules) [6]. Thus, a major assumption that has to made when suggesting that transport to both basolateral and apical plasma membrane domains might occur via cisternal maturation is that the TGN consists of distinct domains, possibly characterized by different lipid/protein compositions. This view is supported by many findings. It has been demonstrated that at least two distinct lipid domains are formed at the TGN level, one of which, containing sphingolipid-cholesterol rafts, has been implicated in sorting to the apical PM [31]. Two different and distinct coats, clathrin-based and lace-like, operate at the TG/TGN level. Indeed, the TGN does not represent a continuous membranous system [32] as has been demonstrated for the Golgi [33]. Two *trans* cisternae participate simultaneously in the generation of TGN tubules and each TGN tubule is connected with its own *trans* cisterna [23]. Of interest, each individual TGN tubule, connected only with its own *trans* cisterna, produces vesicles of only one coat type. Thus, one could speculate that apical/basolateral sorting occurs simultaneously with the maturation of the corresponding carrier within the TG/TGN. Containers directed to the apical and basolateral plasma membrane domains might differ by their lipid composition and their generation might be served by different coats. It is possible that these carriers are formed in physically separated TG/TGN elements. In this case the sphingolipid raft-containing *trans* cisterna connected to TGN tubules would undergo maturation by recycling of resident TG/TGN enzymes through a coat-based (or other) mechanism similarly as described for basolateral constitutive transport. Of course, this idea is clearly speculative and is simply intended to provide the basis for further discussion and experimentation.

5. Concluding remarks

Although the carrier maturation model can reconcile most of the discrepancies between the vesicular model and actual biological observations of transport carried out through different assays, it does obviously need direct confirmation. The present hypothesis is primarily intended to stimulate reassessment of existing data and discussion but also to generate ideas about possible critical experiments aimed at discriminating between these two models. As pointed out above, some aspects of the maturation model can be directly tested. Clearly, the electron microscopic analysis of TGN-PM carriers at different levels will be crucial in attempts to verify the maturation and vesicular models, especially in cells constitutively secreting SA. A particularly powerful but demanding approach will be based on the use of fluorophore-tagged cargo in living cells to study the dynamics of an individual TI, combined with the correlative ultrastructural analysis of the very same TI, especially in combination with the use of a cellular system in which SA are transported from the TGN to the apical PM. It will thus be possible to directly determine how post-Golgi TI are formed.

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