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Inter-organelle communication and membrane shaping in the early secretory pathway

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Abstract

Biomolecules in the secretory pathway use membrane trafficking for reaching their final intracellular destination or for secretion outside the cell. This highly dynamic and multipartite process involves different organelles that communicate to one another while maintaining their identity, shape, and function. Recent studies unraveled new mechanisms of inter-organelle communication that help organize the early secretory pathway. We highlight how the spatial proximity between ER exit sites and early Golgi elements provides novel means of ER-Golgi communication for ER export. We also review recent findings on how membrane contact sites between the ER and the trans-Golgi membranes can sustain anterograde traffic out of the Golgi complex.

Introduction

The spatial compartmentalization of cellular functions is a hallmark of eukaryotic cells. The efficiency by which these cells coped with increasingly complex functions was evolutionarily achieved by the appearance of membrane-bound organelles, which ensure a tight spatial and temporal regulation of intracellular biochemical reactions. A direct consequence of intracellular compartmentation is that each organelle has a well-controlled and well-defined biochemical composition (*organelle identity*). Another consequence is the functional importance of the morphology of these organelles, which is controlled by the tight interaction between membrane mechanics, composition, and cellular biophysics (*organelle shaping*). Situations where organelle identity and/or shape are compromised can have extreme physiological and pathophysiological consequences.

Importantly, the notion that intracellular organelles are independent entities working as distinct functional modules has been refuted in the last decades with the discovery of new means of inter-organelle communication and functional crosstalk. Here, we review some recent advances on how inter-organelle communication controls the shape, identity, and export function of the two main organelles of the early secretory pathway: the endoplasmic reticulum (ER) and the Golgi complex.

General principles of inter-organelle communication

Organelles have different ways of communicating to one another (**Table 1**):

Inter-organelle signaling. This indirect, long-range mode of communication is mediated by signaling molecules and occurs without the physical proximity of the two organelles. On the global cellular scale, organelles can use mechanical cues arising from the cytoskeleton or the extracellular matrix to communicate to one another (mechanotransduction). For example, the Golgi-associated small GTPase Arf1 can be activated downstream of integrin-based cell-matrix adhesions, thereby impacting on Golgi organization and function [1]. Notably, cell contraction regulates Golgi lipid metabolism, COPI-mediated trafficking, and SCAP/SREBP localization by activation of the lipid phosphatase Lipin-1 [2].

Intracellular trafficking. Secretory pathway organelles use membrane-bound transport carriers for long-range communication. Besides their well-established role in shuttling cargoes between organelles, these carriers can also convey additional functions, such as the triggering of mitochondrial fission by Golgi-derived vesicles [3], or the regulation of procollagen export from the ER by ER-Golgi intermediate compartment (ERGIC)-derived membranes [4,5].

Membrane contact sites. Direct inter-organelle communication occurs when the membranes of two or more organelles come to close proximity to one another into a functional contact, commonly referred to as a membrane contact site (MCS). In MCSs, membranes (*i*) are in close apposition tethered by protein-protein or protein-lipid interactions, (*ii*) do not fuse together, and (*iii*) carry a specific biological function [6]. Although the existence of MCSs was already reported in the early days of electron microscopy, it was not until the 1990s when specific biochemical activities and physiological functions were directly linked to these structures (reviewed in [6]). Since then, and particularly during the last decade, our understanding of such mode of inter-organelle communication has dramatically improved. As a result, the field of MCSs has emerged as a major topic in cell biology [6–9].

Inter-organelle transient connections. Finally, it has been suggested that organelles directly communicate to one another by the transient formation of membranous connections between them. Although the existence of such connections in a physiological context remains debated, they have been proposed to serve as a means to tunnel cargo trafficking between Golgi cisternae [10–12], and between the ER and the Golgi/ERGIC [13] for the delivery of large proteins.

Inter-organelle communication for ER export

ER morphology and organization

The ER membrane forms a continuous but intricate network of sheets, helicoidal ramps, and tubules (of ~60 nm in diameter in mammals), which is maintained by a variety of proteins [14,15] and expands throughout the cytoplasm [16–19] (**Figure 1a**). Despite its continuity, the ER membrane presents specialized functional sub-domains, such as ER exit sites (ERES). ERES are enriched in COPII components and serve as departure gates for secretory cargoes [20–22]. Morphologically, ERES are distinct from the rest of the ER, forming single- or multi-budded structures and cup-like structures [23,24] (**Figure 1a,b**). ERES preferentially position on saddle-shaped or highly curved tubular regions of the ER membrane [25]. Although the number and spatial distribution of ERES varies considerably among species, there are generally more ERES in the perinuclear area than in the cell periphery [26].

Preserving a balanced morphology of the ER network is important for cargo export. For example, depletion of Atlastin –a dynamin-like GTPase that tethers and fuses ER tubules into three-way junctions– leads to defects in cargo loading into COPII carriers [27]. These defects are reverted when an Atlastin mutant capable of tubule tethering but not of tubule fusion is expressed. In the absence of Atlastin-mediated tubule tethering, the ER network has a lower connectivity and the membrane is less tense. In addition, the lateral mobility of lipids and proteins is slowed down in Atlastin-depleted cells, leading to less frequent cargo-receptor interactions and cargo export defects [27]. Since both membrane tension [28] and ER network dynamics [17,29] can enhance

protein mobility, Niu et al. proposed that Atlastin facilitates cargo loading in COPII carriers by maintaining the tension of the ER network [27]. This example underscores the importance of ER network morphology for its export functions.

ERES/cis-Golgi communication

A subset of ERES is generally associated to early Golgi elements, such as the *cis*-Golgi cisternae and/or the ERGIC [22] (**Figure 1a**). In yeast, ER arrival sites – ER regions where Golgi-derived COPI vesicles fuse to – enclose the ERES to which they are functionally and dynamically linked [30]. However, despite the growing evidence of the entanglement between ERES and early Golgi elements, it still remains unclear how the ERES structure is dynamically maintained in relation to the overall functional organization of the early secretory pathway.

ER export is altered in different physiological conditions [21]. Particularly, nutrient starvation induces ERES enlargement and enhances the association between ERES and ERGIC. This leads to the translocation of COPII components to the ERGIC, where they form COPII-coated vesicles for autophagosome biogenesis [31].

Of special interest is how inter-organelle communication between ERES and the ERGIC/*cis*-Golgi membranes assists ER export (**Figure 1b**). Procollagens and other large and complex cargoes, such as chylomicrons or apolipoproteins, have challenged the conventional view of how small COPII-coated spherical vesicles mediate export from the ER [13,32]. TANGO1 is an ERES-localized transmembrane protein that connects COPI-coated ERGIC membranes and COPII-coated ERES to allow for efficient procollagen shuttling between these two organelles. TANGO1 forms ring-like structures at ERES [4,33] where it binds both procollagens and COPII components [34]. In addition, TANGO1 recruits ERGIC-derived membranes to the ERES [4,35–38]. Interestingly, ERES-recruited ERGIC membranes seem to have a different function other than in retrograde trafficking: by fusing to the ER, these membranes can locally modify the biophysical properties of the ERES to facilitate procollagen export [5]. A physical model of TANGO1-mediated ER export described how different physical properties, such as membrane tension, dynamically contribute to the formation of procollagen-containing transport intermediates commensurate with the cargo they shuttle [5]. Fusion of ERGIC membranes to an ERES transiently decreases membrane tension, thereby facilitating the elongation of COPII-coated, TANGO1-capped, and procollagen-loaded transport intermediates. How dynamic the TANGO1 rings are and whether or how ERGIC membrane tethering and fusion to ERES is regulated still remain open questions. The proposal that the transmembrane helices of TANGO1 act as membrane shape sensors [39], let us entertain the possibility of a shape-mediated tethering regulation, in which the shape of the elongating membrane would control TANGO1 ring formation and ERGIC membrane tethering and fusion for procollagen export. Altogether, the dual role of TANGO1 as a regulator of ERES structure and export, highlights the important relationship between organelle structure and communication.

Notably, a comparable close contact between ERES and *cis*-Golgi elements exists in *S. cerevisiae* to control anterograde trafficking between these two organelles [40] (**Figure 1b**). Nakano and co-workers proposed a hug-and-kiss model whereby *cis*-Golgi cisternae wrap around and tether ERES (*hug*), after which ER cargo is directionally transferred to the Golgi complex

(kiss). This mechanism does not require the release of COPII vesicles in the cytoplasm for their subsequent Golgi capture [22,40]. Whether such a process creates a transient continuity between these organelles still remains to be elucidated.

Biophysical considerations for transient ER-Golgi connections

The proposal of transient organelle connections poses a major challenge: how could these connections form without causing organelle mixing or collapse? Brefeldin A-induced fusion of Golgi membranes with the ER leads to the rapid and massive absorption of Golgi components to the ER [41]. Early studies indicated that this is not simply mediated by passive diffusion but most likely by a tension-driven directional flow [42]. These data, together with the evidence that the tension of the Golgi membranes is lower than that of the ER [43], seemed to demur the existence of ER-Golgi connections. However, a step towards solving this conundrum arose from the finding that the transmembrane helix arrangement of TANGO1 can act as a lipid diffusion barrier [39]. Diffusion barriers permit minimal mixing of components and prevent a massive and quick absorption of membranes after transient organellar connections. This hypothesis that diffusion barriers help maintain organelle connections is further supported by the recent observations of thicker domains in yeast ER membranes that slow down the diffusion of short transmembrane proteins [44]. These domains are enriched at MCSs, underscoring the possible role of diffusion barriers in ER-Golgi communication.

Overall, there is mounting evidence that there is both direct and indirect functional communication between ERES and early Golgi elements. Such interactions play important regulatory roles in organelle homeostasis and ER export.

Inter-organelle communication for Golgi export

Golgi complex morphology and organization

The Golgi complex is the central organelle of the secretory pathway where cargo maturation and sorting for export take place. The Golgi complex is a polarized organelle, which in mammals is made of stacks of 4–8 cisternae that are laterally connected to form the Golgi ribbon [45]. Golgi cisternae are sac-like structures made of two large parallel flattened membranes (cisternal size ~1 μm ; inter-membrane distance ~30 nm), connected by swollen, highly-curved rims [46] (**Figure 1a**). Such a peculiar membrane morphology guarantees a very large surface-to-volume ratio, thereby presenting some functional benefits: (i) the large surface area of the membrane helps withstand the continuous cycles of transport carrier fusion and fission without a consequential alteration in the biochemical composition of the membrane [47]; and (ii) the difference in curvature between the flat sheet-like part of a cisterna and its highly curved rim provides a morphological cue to organize in space and time the different Golgi functions. Physical modelling indicated that membrane budding effectors help maintain cisternae flat by stabilizing the highly curved rims [48], hence highlighting the connection between the export capabilities of the Golgi complex and its shape.

There is increasing evidence that Golgi-resident proteins (such as glycosylation enzymes) are enriched in the flat parts of the Golgi membranes, whereas export machinery localizes to the highly curved rims [48–52]. Electron tomography showed the accumulation of S-palmitoylated cargoes at the rims of Golgi cisternae [52]. A separate study using super-resolution microscopy confirmed that glycosylation enzymes and the budding machinery segregate along the radial direction of the cisternae [53], backing the idea of a lateral segregation of Golgi functions. Various mechanisms contribute to dynamically organize the different reactions occurring in the Golgi membranes, including lipid-mediated, curvature-mediated, calcium-mediated, or receptor-mediated mechanisms (reviewed in [54–57]). While these studies emphasize how Golgi morphology impacts its secretory function, we review below how morphology is also a key aspect of Golgi inter-organelle communication, focusing on ER-Golgi MCSs and their role in export from the trans-Golgi network (TGN).

ER-Golgi MCSs

MCSs enable lipid transfer for TGN export. Golgi lipid homeostasis is preserved by non-vesicular transfer of lipids, which takes place at ER-Golgi MCSs (**Figure 1c**). These contacts form by the tethering of ER-localized VAP-A/B to a number of TGN-anchored lipid transfer proteins, such as OSBP or CERT, which are recruited to the TGN by binding to PI(4)P by their pleckstrin homology domains [8,58]. Notably, this lipid-protein binding is sensitive to cytosolic pH, which can be for instance modulated in response to nutrient (glucose) availability, thus enabling signal-mediated communication at the Golgi membranes for efficient cargo sorting and export [59]. ER-Golgi MCSs directly control the levels of sterols, sphingolipids and PI/PI(4)P in these organelles [8,58], and these lipids are crucial for cargo export from the TGN [55,60–63]. In particular, altering the sphingolipid metabolism at the Golgi membranes causes a massive cisternal curling into shapes with little rim area [48,51]. These shape changes are accompanied by global inhibition of cargo export from the Golgi complex [61], glycosylation defects [51], and mis-localization of Golgi proteins [48,51,64], highlighting the importance of MCS-regulated lipid homeostasis for organelle shaping and transport carrier biogenesis at the TGN.

MCSs contribute to transport carrier biogenesis. Dynamic ER-Golgi MCSs are needed for the export of a subset of cargoes from the TGN [65–67]. Both the depletion of ER-Golgi MCS components and contact site immobilization inhibit the formation of a specific class of transport carriers named carriers of the TGN to the cell surface (CARTS) [65,66]. Notably, super-resolution microscopy revealed that CARTS form at TGN regions that are adjacent to ER-Golgi MCSs, opening the possibility that ER-Golgi communication via MCSs could directly facilitate membrane budding and/or fission for CARTS formation [66] (**Figure 1c**). Interestingly, in yeast, peak-shaped ER membrane regions of very high curvature were observed at ER-plasma membrane MCSs and appear to enhance the lipid transfer rate [68,69]. The formation of these membrane regions of high curvature is mediated by tricalbins (extended-synaptotagmins, in mammals), which can also localize at ER-Golgi MCSs where they contribute to ceramide transfer [70]. Whether similar high curvature membrane peaks are present at ER-Golgi MCSs for transport carrier formation at the TGN remains to be investigated (**Figure 1c**).

Biophysical considerations of MCSs. MCSs play direct roles in determining organelle fission sites and initiating membrane constriction [71–73]. Short distances between membranes impose a series of mechanical effects and constraints, such as the limitation in the amplitude of membrane fluctuations/movements (e.g. membrane pinning [74]), or steric hindrance that prevents proteins with large cytosolic domains to enter the contacts [75,76]. It is therefore becoming increasingly clear that MCSs have a pleiotropic effect in the way they regulate organelle dynamics and function.

Summary and future perspectives

Since the pioneering discoveries by George Palade and colleagues, we have learned how the ER and the Golgi complex are intimately linked by their shared function in intracellular trafficking and protein secretion. Beyond that, new interaction modes in the early secretory pathway are just beginning to be uncovered. Some examples, which we highlighted in this review, are the close contacts between ERES and early Golgi elements as well as ER-Golgi MCSs. These investigations herald a whole new wave of research in inter-organelle communication, further establishing its often unexpected but central role in cell homeostasis.

Defining the molecular and biophysical basis of inter-organelle communication will pave our way to better understand the secretory pathway in health and disease. Combining novel approaches in fluorescence microscopy [17,29,77], and/or cryo-electron tomography [68,69], with tools to specifically monitor organelle contacts – such as fluorescence complementation [66,73] or FRET-FLIM-based tools [78] – will help to obtain ultra-highly resolved spatio-temporal maps of organelle connections. Moreover, *in vitro* reconstitution of inter-organelle contacts is a promising approach to study the fundamentals of these structures [79]. Pooled CRISPR/Cas9-mediated loss of function genetic screens provide unbiased ways of finding novel components involved in these processes both in 2D cell cultures [80] and in 3D tissue organoids [81]. Finally, a physical understanding of the principles governing how inter-organelle communication affects organelle shape and identity will be an added value to improve our holistic understanding of the early secretory pathway.

Conflict of interest

The authors declare no conflict of interest.

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TABLE 1. Inter-organelle communication in the early secretory pathway.

Inter-organelle communication mode	Contact between organelles?	Membrane fusion?	Examples
Inter-organelle signaling	No	No	<ul style="list-style-type: none"> • Cell-matrix adhesions control Golgi function and organization by Arf1 activation [1] • Mechanotransduction at the Golgi complex mediated by Lipin-1 and SREBP [2]
Intracellular trafficking	No (mediated by organelle-derived membrane-bound transport carriers)	Yes (of membrane-bound intermediates, but not of organelles)	<ul style="list-style-type: none"> • COPI- and COPII-coated transport carriers
Membrane contact sites	Yes	No	<ul style="list-style-type: none"> • ER-Golgi MCS mediate Golgi lipid homeostasis [8] and CARTS formation [19,20]
Inter-organelle transient connections	Yes	Yes	<p>Not well established.</p> <ul style="list-style-type: none"> • Heterotypic fusion between Golgi cisternae [11,12] • ER-ERGIC tunnels for procollagen export [13]

FIGURE 1

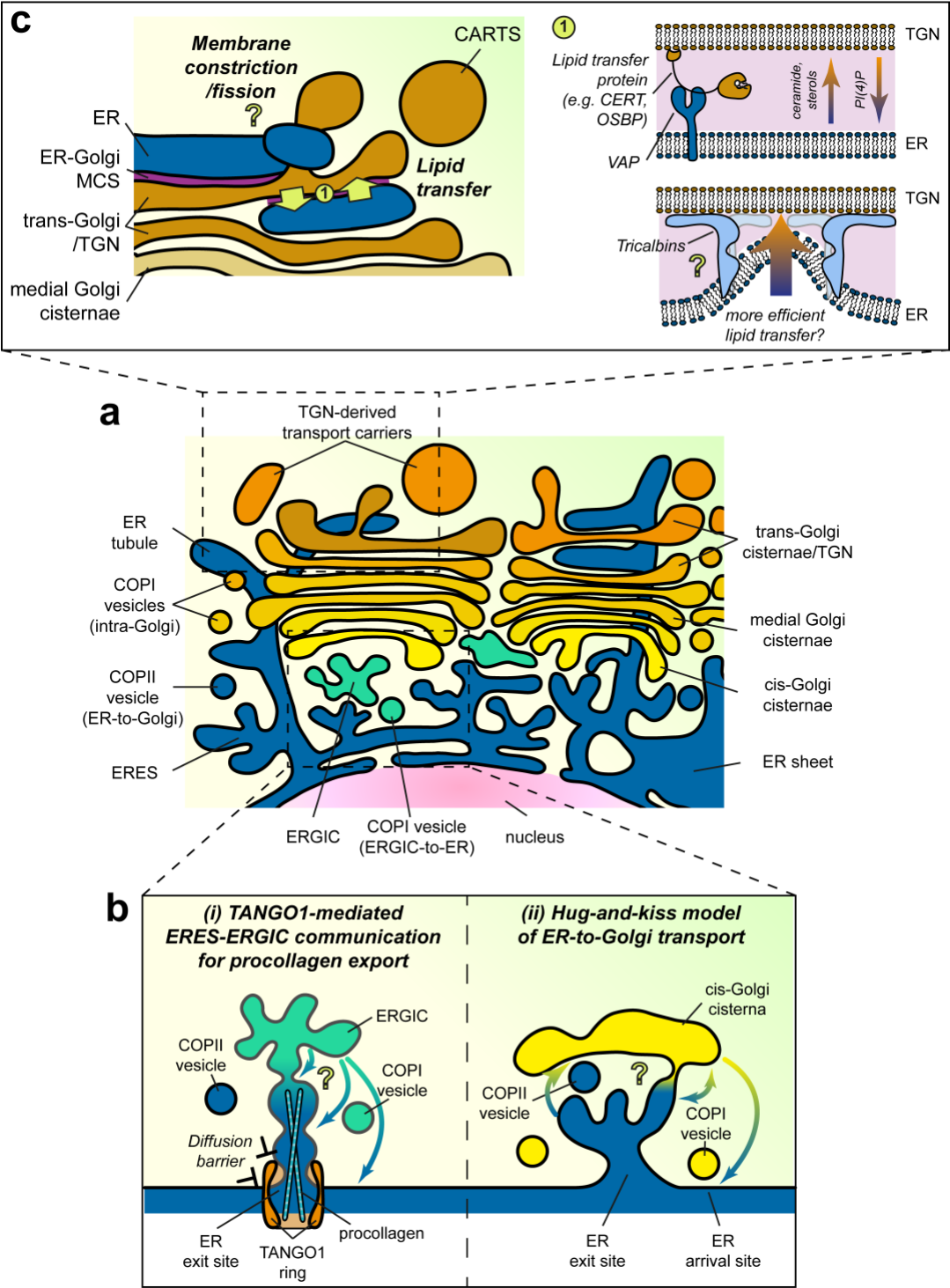


Figure 1. Inter-organelle communication and membrane shaping in the early secretory pathway. (a) Schematic representation of the membrane-bound organelles involved in the early secretory pathway in mammalian cells. **(b)** Two modes of communication between ERES and early Golgi elements. *(i)* Mechanisms of ERES/ERGIC communication for procollagen export in

mammalian cells. A TANGO1 ring (orange) links procollagen (cyan-striped poles) to COPII coats (not shown). TANGO1, by binding to a tethering complex (not shown), recruits ERGIC/COPI membranes (light green) to a nascent transport intermediate. Fusion of these membranes triggers carrier growth. The precise fusion site is yet to be revealed. Finally, TANGO1 creates a diffusion barrier at the carrier base to prevent membrane mixing (ER membrane, black lines; COPI/ERGIC membrane, gray lines). *(ii)* Hug-and-kiss model for proximity-based ER-to-Golgi transport in yeast. A dynamic contact between ERES (blue) and cis-Golgi cisternae (yellow) enhances the efficiency of anterograde trafficking. Whether COPII vesicles are fully formed and travel the short distance within the contact, or alternatively a transient fusion of the ERES with a cis-Golgi cisterna occurs remains unresolved. An ER arrival site where Golgi-derived COPI vesicles fuse to the ER is shown proximal to the ERES. **(c)** ER-Golgi MCSs contribute to TGN export. Membranes of the ER (blue) and the trans-Golgi cisternae/TGN (orange) come into close apposition in ER-Golgi MCSs (magenta). These MCSs are established by ER tethers (VAP proteins) and Golgi-attached lipid transfer proteins. They can facilitate transport carrier formation by regulating the lipid composition of the TGN membrane. It is possible that MCSs could additionally play a direct role in membrane shaping (e.g. by recruiting curvature-inducing proteins such as tricalbins) or in constricting the neck of a transport carrier (such as CARTS, in orange) for its fission.