TANGO1 marshals the early secretory pathway for cargo export

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Abstract

TANGO1 protein facilitates the endoplasmic reticulum (ER) export of large cargoes that cannot be accommodated in 60 nm transport vesicles. It assembles into a ring in the plane of the ER membrane to create a distinct domain. Its lumenal portion collects and sorts folded cargoes while its cytoplasmic domains collar COPII coats, recruit retrograde COPI-coated membranes that fuse within the TANGO1 ring, thus opening a tunnel for cargo transfer from the ER into a growing export conduit. This mode of cargo transfer bypasses the need for vesicular intermediates and is used to export the most abundant and bulky cargoes. The evolution of TANGO1 and its activities defines the difference between yeast and animal early secretory pathways.

Introduction

The ER-Golgi secretory pathway is a manufacturing, processing, and distribution system where cargo proteins are synthesized (ER) and then processed in membrane-bound cisternal layers (Golgi stack), before being delivered to their final destinations. Approximately 30% of all proteins enter the secretory pathway.

Pioneering genetic screens in *Saccharomyces cerevisiae* and in vitro reconstitutions using mammalian systems have informed our understanding of conserved mechanisms in the secretory pathway [1,2]. However, we lacked an appreciation of metazoan-specific evolutionary adaptions that allow for tremendous variations in cargo size, volume, secretory timing, and cell-specific specialised secretory activities [3].

A secretory function for TANGO1 was first discovered in one of the first metazoan genome-wide RNAi screens [4]. This screen selected Drosophila genes whose depletion prevented secretion of the engineered soluble cargo protein ss-HRP (horseradish peroxidase with a signal sequence) and altered the organization of the Golgi apparatus. Gene hits were named TANGO (Transport and Golgi Organization) and many were metazoan-specific secretory genes that were not identified in yeast [4].

We describe here recent mechanistic insights into bulky secretory cargo export in the early secretory pathway, focusing on the central role of the TANGO1 family of proteins in organising and scaffolding membranes at the ER-ERGIC interface. We highlight our current understanding of how membrane-sculpting and tethering

machinery are utilised and repurposed to allow for the dynamic and efficient export of bulky, high-volume cargoes.

ER exit site (ERES) machinery and COPII budding

COPII-coated vesicle assembly has been reviewed extensively [5]. The COPII coat machinery consists of five cytosolic proteins: Sar1, Sec23, Sec24, Sec13 and Sec31. In addition, there is an ER-resident transmembrane protein, Sec12, and a membrane associated protein, Sec16. In cells, Sec23 and Sec24 are found in constitutive heterodimers, whereas Sec13 and Sec31 are found in stable heterotetramers of two subunits of each protein.

The process of COPII-dependent budding is initiated when the small cytoplasmic GTPase Sar1 is recruited to the ER membrane by the GTP exchange factor (GEF) Sec12. Sar1-GDP is cytosolic and dormant, but when bound to GTP, Sar1 activates and exposes an amphipathic N-terminal α-helix that inserts shallowly into the ER membrane [6]. Membrane-bound activated Sar1 directly recruits the Sec23–Sec24 heterodimer. Sec24 binds to membrane cargo proteins and cargo receptors to concentrate them into the forming vesicle. Together, Sar1 with a cargo-loaded Sec23–Sec24 dimer is termed the ‘pre-budding complex’ ready to recruit Sec13–Sec31 to complete vesicle formation. The Sec13–Sec31 complexes polymerize to assemble into an outer coat layer that shapes the membrane, forming a bud enriched in cargo molecules. Extruded membrane is separated from the donor ER membrane by fission, to form an intact spherical vesicle roughly 60 nm in diameter.

These stereotypically sized vesicles might be sufficient to account for the secretory cargoes and requirements of a yeast cell, but they are unsuited to the challenges imposed by much larger metazoan cargoes [3,7].

Metazoan secretory cargoes

Metazoan-specific cargoes include collagens, the main components of animal extracellular matrix (ECM), which fold into rigid triple helices in the ER that can extend up to 300–400 nm [8] and also much larger complexes, such as apolipoprotein particles and giant cuticular proteins of insects. These cargoes are far too large to fit inside conventional ~60 nm COPII-coated vesicles as described in yeast cells.

In addition to size-based constraints, metazoan cells have also had to develop the capacity to secrete enormous amounts of cargoes constitutively under specific conditions. This high-volume export needs efficient physical organisation of secretory organelles for rapid material transfer between them and also for maintaining organelle homeostasis (see e.g. [9]).

To manage these large volumes of cargoes of varying size and complexity, metazoan cells have evolved a different early secretory pathway organisation [10]. COPII-dependent carriers are formed at specialised domains of the ER known as ER exit sites (ERES) and metazoans have developed a characteristic shape and organization of ERES.

ERES

ERES function as departure gates for secretory traffic. They are specialised high-curvature subdomains of the ER, [11] that are depleted of bound ribosomes and enriched in COPII-coat proteins. Mammalian ERES are stable clusters of 2–6 COPII buds [12], of limited mobility.

Drosophila and mammalian ERES membranes consist of an intertwined tubule receptacle that can extend away from the ER, while still connected to ER by a narrow neck of ~40nm in diameter [13–16]. ERES-localized COPII proteins could drive the formation of small vesicles and may also help to generate polymorphic transport carriers. Metazoan ER-to-Golgi carriers can be pleomorphic and remain uncoated [17].

Extra functionalities have been acquired by metazoan ER export sites to enhance export capabilities. Remarkably, the TANGO1 family of proteins add the following features to metazoan export events. They:

1. Connect cargoes such as collagen to COPII proteins.
2. Are required for the ER export and secretion of bulky cargoes.
3. Cluster and constrain ERES machinery and membrane within a defined area; recruit and organise downstream membrane compartments (such as ERGIC or *cis*-Golgi elements) at the ERES.
4. Are ERES-resident and can change COPII lattice assembly to accommodate metazoan cargoes of varying size and shape.
5. Can dynamically regulate ERES assembly and disassembly.

In the following sections, we describe how each of these activities are carried out by the TANGO1 family of proteins and how these discoveries have made the processes of bulky, or high-volume, export amenable to molecular analyses.

TANGO1: a metazoan family of proteins

TANGO1 first appeared in metazoa and is expressed in almost every metazoan with the notable exception of nematodes, where the gene appears to have been lost. In invertebrates, TANGO1 is the only gene in the family, but vertebrates have acquired an expanded family of TANGO1-related proteins. The human genome encodes 10 genes for MIA/cTAGE family proteins that share 34%–45% amino acid identity and 47%–59% cDNA sequence homology. In mammals, the gene family is also known as the MIA (melanoma inhibitory antigen) family.

The domain architecture for TANGO1 is maintained across family members (Figure 1). In the ER lumen, there is an N-terminal SH3-like domain, and an extended region of high predicted intrinsic disorder. There are two hydrophobic helices, one inserts into the lumenal leaflet of the ER membrane, while the second one is a transmembrane helix. In the cytoplasm, there are two regions predicted to adopt a coiled-coil fold and a poorly conserved C-terminal proline-rich domain (PRD) with periodic occurrences of consecutive prolines. The PRD in TANGO1 can bind up to seven Sec23A molecules and Sec16, while the cTAGE5 PRD can bind up to five Sec23A (see the cytoplasmic C-terminal region of proteins in Figure 1).

TANGO1 (MIA3) and TANGO1-like (TALI or MIA2), are most reminiscent of Drosophila TANGO1. The other genes contain the SH3 domain and varying lengths of the ER-lumenal portion, or the cytoplasmic portion including a transmembrane domain.

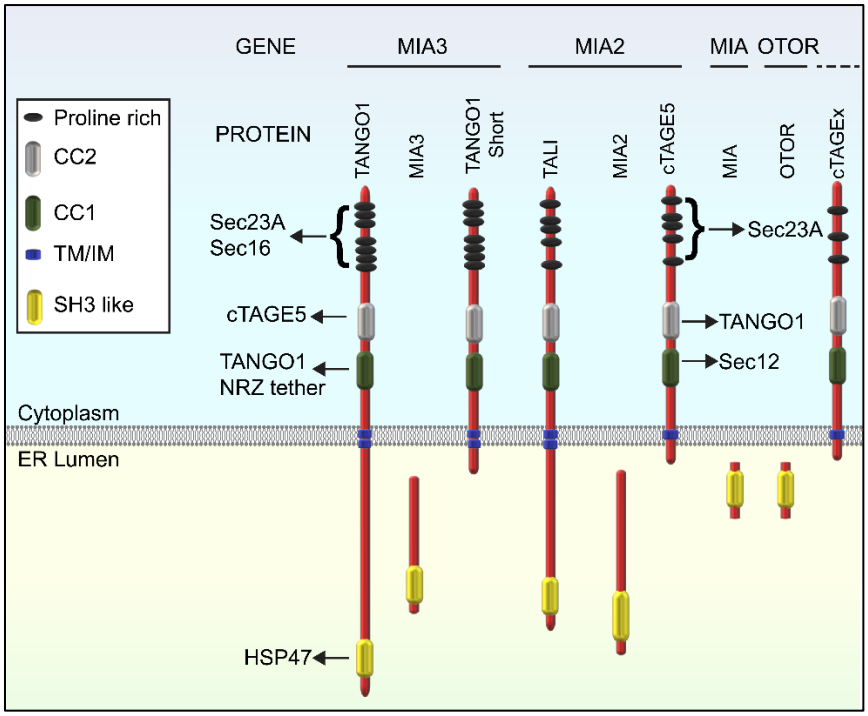

Figure 1: The MIA family in mammals. Schematic representation of mammalian TANGO1 (MIA/cTAGE) family proteins. Full length TANGO1 or TANGO1-like (TALI) consists of an ER-lumenal part, with extensive regions of high intrinsic disorder and an SH3-like domain. The cytoplasmic part, contains coiled coils (CC1 and CC2) and a C-terminal proline-rich domain. Other isoforms consist of exclusively ER-lumenal portions (MIA and Otoraplin) and transmembrane cytoplasmic forms, TANGO1-Short, cTAGE5 and several other predicted cTAGEx proteins (where x is 2, 4, 6, 8, 9, and 15). The TANGO1 SH3-like domain binds to HSP47, which recruits fully folded, export competent collagen. Via its CC1, TANGO1 binds itself or the NRZ tether. Via the CC2 domains of TANGO1 and cTAGE5 bind to each other. On the other hand, via its CC1, cTAGE5 interacts with the GEF Sec12. Via proline-rich domains (PRD), TANGO1 interacts with COPII machinery Sec23A and Sec16.

TANGO1 interacts with ERES machinery

TANGO1 is an ERES-resident protein, recruited to exit sites by interacting with multiple ERES-proteins. Drosophila TANGO1 interacts with Sec23A, Sec16, and Sar1. These functions have been partitioned between TANGO1 and cTAGE5 in vertebrates; both TANGO1 and cTAGE5 interact with Sec16A and Sec23A while cTAGE5 alone retains the ability to interact with Sec12 (Figure 1) and therefore with Sar1 [18–21].

Vertebrate TANGO1 and cTAGE5 also interact with each other via another cytoplasmic coiled coil. This coiled-coil region is well conserved through evolution and it is not clear what functions or interactions it mediates in invertebrate TANGO1 where there is no cTAGE5. TANGO1 also self-associates via a cytoplasmic, membrane-proximal coiled domain [22,23].

TANGO1 interacts with cargo

In the ER lumen, mammalian TANGO1 uses an SH3-like domain to interact with the chaperone HSP47 (SerpinH1), to couple procollagen triple helix formation with ER exit [24]. The mechanism of export-competent cargo selection remains unclear in invertebrates, which do not express an HSP47-like protein.

TALI co-immunoprecipitates with ApoB, suggesting that TALI interacts with apolipoprotein particles, though it is not known whether the interaction is direct or via an intermediate adaptor analogous to HSP47 that connects TANGO1 to triple helical collagen [25]. Future experiments are needed to explore the cargo selectivity of different TANGO1 family proteins.

Altogether, TANGO1 connects challenging metazoan cargoes to ER export machinery.

TANGO1 family is required for bulky cargo secretion

TANGO1 is required for collagen export from the ER in Drosophila, mice, Zebrafish and Medaka [3]. Biallelic mutations in the TANGO1 gene lead to severe dentinogenesis imperfecta, short stature, and various skeletal abnormalities, consistent with a key role for TANGO1 in collagen secretion [26]. A complete loss of TANGO1 expression in humans is embryonically lethal, associated with an absence of bone mineralization [27].

Hepatocyte-specific deletion of cTAGE5 leads to severe fatty liver and hypolipemia in mice, likely due to impaired secretion of very-low-density lipoproteins (VLDL), from the liver. An alternate protein, formed from the same gene that codes for cTAGE5, known as TALI (TANGO1-like or Mia2), has a tissue-specific expression and is required to export bulky lipid particles such as chylomicrons or VLDLs to regulate cholesterol metabolism [25,28,29].

It appears that TANGO1 is required for efficient secretion of high-volume cargoes, not only large cargoes. In Drosophila, TANGO1 expression correlates with the secretory requirement of the tissue [30]. cTAGE5 deletion in murine pancreatic β cells impairs proinsulin trafficking, perhaps because insulin is retained in the ER in the absence of cTAGE5 [31]. It is also possible that a lack of cTAGE5 causes ER stress that will impact the capacity of the ER to produce large amounts of proinsulin. Future

experiments will be needed to show association of proinsulin with a member of the TANGO1 family. Depleting TANGO1 causes several ECM proteins to be retained in the ER, including BM-40-SPARC [32], Laminin [33], Perlecan [34], and Papilin [35].

Though TANGO1 binds to ER lumenal cargo and cytoplasmic inner COPII coat proteins, two observations set it apart from conventional cargo receptors. First, TANGO1 binds to Sec23A, instead of Sec24 like most other receptors. Second, TANGO1 does not leave the ER with the departing cargo, as does every other cargo receptor.

Altogether, TANGO1 is present at ERES and is required for the ER export and secretion of complex metazoan cargoes. It is retained at ERES during cargo export and therefore is involved in organising export machinery there in a way that has proven key to our understanding of how the early secretory pathway is organised in animals.

TANGO1 forms a ring around COPII/ERES

Depleting TANGO1 in Drosophila from either the fat body or salivary glands, reduces ERES size, suggesting that TANGO1 contributes to ERES architecture. At the ER, TANGO1 assembles into a ring-like filament in the plane of the ER membrane, which encircles and physically constrains COPII components. Super-resolution microscopy in human cell lines [36], in Drosophila fat bodies [21] and live imaging of GFP-tagged TANGO1 in Drosophila larval salivary glands [23] showed linear, filamentous arrays of TANGO1 surrounding exit sites.

At ERES, TANGO1 filaments are held together by protein–protein interactions between TANGO1 and cTAGE5. Individual proteins in the filament bind to the peripheral inner coat proteins as a COPII lattice assembles into a hemispherical bud. This binding effectively reduces the line energy of the COPII coat, suggesting that TANGO1 filaments act as linactants to stabilise an incomplete COPII lattice. Notably, a biophysical model of TANGO1-mediated cargo export showed that TANGO1 rings can arrest the growth of standard COPII carriers, thereby effectively stabilizing open necks at the ER-ERES interface for efficient cargo loading [37].

These abilities of TANGO1 satisfy our fourth criterion, by clustering ERES machinery for secretory traffic of challenging cargoes.

TANGO1 recruits membrane

A key difference between the yeast and metazoan early secretory pathways is the presence of an anastomosis of membranes that appear tethered at metazoan ERES, physically contiguous with the ER, but maintained as a distinct sub-compartment [13,14,38]. To generate this interwoven, tubular network extending from ER, membranes need to be tethered at the ERES and held in place as they fuse with each other and interconnect. Membrane tethering plays a key role in organelle biogenesis and maintaining compartment identity. TANGO1 interacts with a multisubunit tethering complex, called the NRZ tether (NBAS, RINT1, ZW10), that tethers retrograde COPI-coated membrane to the ER [22]. In Drosophila fat body extracts, TANGO1 interacts with post-ER membrane-localised proteins GM130, GRASP, and Rab1. These interactions allow TANGO1 to effectively hold together the ER and downstream membranes (Figure 2). Accordingly, loss of TANGO1 causes ERES-Golgi uncoupling in Drosophila [21] and ERES-ERGIC uncoupling in mammalian cells [39].

TANGO1 reorganises COPII lattices

The COPII machinery appears to provide a collar to form a narrow neck connecting the ER with ERES/ERGIC membranes. This requires that the COPII machinery is able to assemble into at least two different lattice structures - one the conventional spherical shape and the other more cylindrical.

Beyond COPII, there are two ways by which TANGO1 might control the shape of the nascent carrier. First, a ring comprised of TANGO1 and cTAGE5 controls the location of Sar1 recruitment and the orientation adopted by Sar1-GTP when its amphipathic helix is inserted into the ER membrane. Sar1 orientation, in turn, controls local membrane curvature and/or shape of a bud at the ERES.

A second way stems from the multiple triple-proline motifs at the C termini of TANGO1 and cTAGE5, which bind the gelsolin-like domain of Sec23A [40] and are shared among other Sec23-binding proteins including the scaffolding protein Sec16. These proline residues appear to bridge adjacent inner coat subunits, controlling inner coat lattice shape. Together, these two functionalities could serve to change a COPII lattice from a spherical shape to a cylindrical collar. COPII lattices can therefore participate in at least two distinct self-assembly reactions forming either a spherical coat or a cylindrical collar, with TANGO1 and cTAGE5 controlling the equilibrium between these two states (Figure 2).

In addition to the altered lattice shape, there needs to be careful temporal control of coating to maintain the COPII collar as long as necessary and prevent it from uncoating prematurely. COPII uncoating is linked to GTP hydrolysis by Sar1, which is stimulated by the GTPase-activating protein (GAP) Sec23. Sec23 activity in turn is stimulated up to tenfold when Sec31 binds to it. As the PRDs of TANGO1 and cTAGE5 bind to Sec23 [18,19], they will competitively inhibit the binding of Sec31 to Sec23. A ring of TANGO1 assembles around budding structures at the ERES, and therefore it will exert a tight control over the timing and activity of Sar1 GTP hydrolysis at the base of a COPII bud. Once the bud grows beyond a certain size, TANGO1 PRDs can no longer access distal Sec23A. Now the 'far' end of the coat begins to disassemble and the naked carriers are free to fuse to other membranes in the vicinity.

By this combination of strategies, TANGO1 can ensure that the COPII lattice remains as a collar at the base of membranes extruded from the ER and fused into the intertwined tubular receptacle as seen at mammalian ERES (Figure 2).

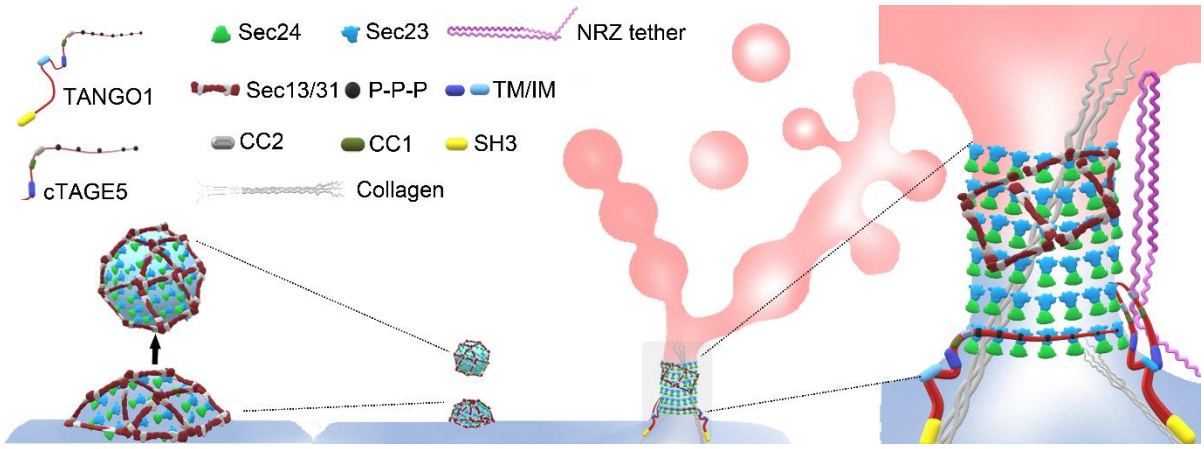

Figure 2: ER exit site with COPII proteins as coats and collars. COPII proteins assemble in two layers into a conventional coated vesicle (left). TANGO1 family reorganise COPII proteins to generate a collar between the ER (blue) and a downstream COPI-coated ERGIC compartment (peach). TANGO1 proteins bind via triple proline motifs in their PRDs (shown as black dots) to Sec23. The COPII collar-TANGO1 module is used to export bulky or high-volume cargoes, such as triple helical collagen (shown in grey).

Phase separation promotes layering of ER-ERGIC-Golgi

A final speculative aspect could be ascribed to how TANGO1 organises ERES. Recent advances in our understanding of cellular physical states have revealed that liquid-like "membraneless" organelles can self-assemble based on "liquid-liquid" phase separation (LLPS) [41]. Proteins can form a biomolecular condensate together with lipid vesicles [42]. Recent proposals have suggested that even membrane-bound

organelles are templated by analogous phase separations of their surface proteins into largely planar liquids, akin to liquid crystals. LLPS may therefore underlie the formation of a matrix that gives rise to the multi-layered architecture of the secretory pathway [43]. Adherent liquid crystal-like phases of proteins that form a matrix will surround and enclose membranes of individual layers. A recent model posits that the matrix consists of layered planar liquid crystal arrays of golgins, which are Golgi-localized transmembrane or membrane-associated proteins with long cytosolic coiled-coil regions [43,44].

TANGO1 and cTAGE5 share many architectural features with golgins, transmembrane proteins with long cytosolic coiled-coil regions. Extending the hypothesis, we suggest that physico-chemical interactions of golgins and the TANGO1 family specify the topology and dynamics sequential compartments in the ER-Golgi stack. By self-assembling to form a dynamic scaffold they could help template the early secretory pathway.

At the ERES, locally condensed environments are generated through collective phase separation of proteins including Sec16, Sec12, TANGO1 and cTAGE5 [45]. The high local concentration of ERES proteins including COPII coats in these condensates, could catalyse spatially restricted biochemical reactions and interactions to facilitate ERES building.

Dynamic control of ERES

ERES are disassembled and reassembled during mitosis. In mammalian cells, TANGO1 phosphorylation reduces its interaction with Sec16, leading to ERES disassembly. Casein kinase 1 (CK1) constitutively phosphorylates TANGO1 while protein phosphatase 1 (PP1)-mediated dephosphorylation decreases during mitosis. Thus, coordinated cycles of TANGO1 phosphorylation and dephosphorylation regulate ERES organisation during the cell cycle [46]. The putative phosphorylation sites, conserved in vertebrates, do not seem to be conserved in Drosophila and it remains to be seen whether a similar process occurs in invertebrates that is controlled by a different kinase.

TANGO1 is upregulated in response to increased cargo load requirements. For example, the ER-resident transcription factor BBF2H7 (CREB3L2) is highly expressed in chondrocytes [47]. Bbf2h7 increases expression of Sec23A, Sec24D and TANGO1

to stimulate ER to Golgi trafficking of the sizable ECM protein load, particularly in cartilage. Given its important role, it is not surprising that Bbf2h7$^{-/-}$ mice are phenotypically similar to Mia3$^{-/-}$ mice [48,49].

In short, TANGO1 dynamically regulates ERES assembly and disassembly.

TANGO1 membrane helices form a lipid diffusion barrier

Having delineated how TANGO1 can tether membranes and reorganise ERES machinery to bring together the ER and the ERGIC, we are presented with an important issue, how are the two organelles kept distinct biochemically? After all, when retrograde ERGIC/Golgi membrane elements are recruited to an ERES, their fusion needs to be constrained and stabilised during cargo transfer out of the ER, while maintaining the compositional identities of the individual compartments.

Diffusion barriers control mixing of components to prevent a rapid mixing of organellar membranes during transient connections. It will be interesting to study how the existence of such diffusion barriers can control the local dynamics of membrane tension at ERES (see also next section).

The unusual membrane helix organisation of TANGO1 is composed of one membrane-spanning helix and another inserted into the inner ER membrane leaflet. Reconstituting these helices in model membranes revealed that they reduce the flow of lipids at a region of defined shape [50]. TANGO1 ring assembly acts to reduce the flow of lipids, and probably also transmembrane proteins, at the boundary between the ER and downstream membranes fused at the ERES.

Controlling membrane tension

Extraction and addition of membrane are processes that influence, and are in turn influenced by, the membrane tension of donor and acceptor membrane organelles. When a membrane is stretched, the high lateral tension works against membrane bending, so membrane deformation is attenuated; it is therefore less likely that membrane will be pinched away from it. Conversely, it is easier to fuse incoming membrane intermediaries [51]. Membrane tension regulates basic functions of the plasma membrane including rates of endocytosis, but much less is known about intracellular membranes. Different organelles have different membrane tension and

small tension gradients in the plane of the membrane are sufficient to power rapid movements by membrane flow [52,53].

Biophysical modeling of collagen export from the ER suggested that TANGO1 self-organisation around COPII coats, stabilises shallow buds at physiological ER membrane tensions [37]. Shallow buds facilitate TANGO1-mediated procollagen recruitment, packaging, and ERGIC/COPI membrane recruitment to the ERES. However, as cargo continues to be packaged into the nascent carrier, these shallow buds are too small to accommodate bulky cargo. The membrane is stretched at a high tension. Transient reduction of membrane tension might be mediated by fusing ERGIC/COPI membranes to the ER, allowing the buds to grow from shallow to elongated pearled transport intermediates of any size required to export bulky cargo. This dynamic computational model predicted that TANGO1-dependent, procollagen-containing transport intermediates are shaped as pearled tubules. These predictions have been recently confirmed by the visualization of cargo-containing pearled tubular structures at ERES by correlated cryo-SIM/FIB-SEM microscopy [14]. The biophysical model also showed that formation of long, procollagen-filled transport intermediates is promoted not only by tension reduction but also by the application of pulling forces. A possible force-generating agent is the microtubule-associated motor dynactin, which has been visualized in close proximity to cargo-containing transport intermediates at ERES [14]. Secretion rates are only slowed about two fold in cells in the absence of microtubules, so pulling may increase secretion efficiency, but procollagen does not necessarily require an intact microtubule network to leave the ER [54]. The extent of the force-dependent transport intermediate formation mechanism in procollagen export remains to be elucidated.

Cellular control of membrane trafficking by membrane tension is challenging to study experimentally and remains poorly understood. New reagents are being made available to visualise membrane tension in different organelles [55,56] and could describe how tension correlates with cell state and with cargo flow. Ultimately, such studies can provide information both about how tension is controlled dynamically and, conversely, how tension itself feeds back into regulating membrane traffic.

Pathophysiologies associated with the TANGO1 family

With such a central role in organising the metazoan secretory pathway to secrete bulky cargoes, it comes as no surprise that the TANGO1 family is implicated in several human pathophysiologies.

Zika virions inside the ER, associate into extensive paracrystalline lattices that can transit in their entirety out of the ER, all the way to the plasma membrane [57]. These assemblages present similar challenges to the ER export machinery as bulky cargoes like collagens; COPII proteins are required to transport large viral conglomerates, but it remains unclear how the proteins are utilised. Interestingly, in Zika-infected cells, the transcription factor CREB3L1 is upregulated. As discussed earlier, CREB3L transcription factors control the expression of cTAGE5, TANGO1, Sar1 as well as Sec24D. All these factors are also upregulated in flavivirus-infected cells [58].

SARS-CoV-2, the causal agent of Coronavirus Disease 2019 (COVID-19) pandemic, is a positive-sense single-stranded RNA virus and it induces massive remodeling of host cell secretory membranes. Multiple SARS-CoV-2 proteins interact with the TANGO1 family. ORF7B, M, ORF3B, ORF6, ORF8, E and S have been identified as TANGO1 interactors. NSP3, NSP4, E, M, ORF7B interact with cTAGE5 [59]. Flaviviruses critically depend on the cellular secretory pathway for virion formation, maturation and release [60]. Viral proteins could repurpose the ability of TANGO1 family proteins to remodel secretory membranes, though the mechanisms of this remodeling remain unknown.

Defects in collagen secretion affect every organ system and are the pathophysiological hallmark of collagenopathies (connective tissue disorders) and pathological fibrosis (dysregulated scar tissue formation). A hallmark of liver cirrhosis is fibrogenesis: secretion and deposition of excess extracellular matrix (ECM) proteins, particularly collagen I, by hepatic stellate cells (HSCs). TANGO1 is critical for fibrogenesis in mice and is specifically upregulated in HSCs in response to pro-fibrotic stimuli in a UPR-dependent manner [61].

TANGO1 family members have several tumour-related functions, but their mechanistic roles in malignancies remain poorly elucidated. MIA2 has contrasting functions in different pathologies; it is induced in liver fibrosis or cirrhosis, acts as a suppressor in liver cancers, and is associated with a loss of chemosensitivity in pancreatic cancers. TANGO1 expression has been correlated with tumour suppressive activities in malignant melanoma and colorectal cancer [62].

Several genome-wide association studies have described a close association between TANGO1 and coronary artery disease, myocardial infarction or accelerated atherogenesis observed in rheumatoid arthritis [63,64].

Using population- and single-cell transcriptomics, as well as long-term lineage tracing to follow murine dermal fibroblasts during physiological aging, it was observed that TANGO1 expression is anti-correlated with age, with reduced TANGO1 expression in fibroblasts from older individuals [65].

Conclusions

The TANGO1 family is the archetype of metazoan-specific proteins that have evolved to expand the repertoire of cargoes that the secretory pathway can accommodate. Reorganization of ERES membranes and activities by TANGO1 improves the efficiency of ER export. It is likely that the more challenging the cargo, the greater the role of TANGO1 in its export. Bulky cargoes such as procollagens or highly abundant cargoes such as antibodies or overexpressed proteins will display the greatest dependence on TANGO1 for their ER export. Therefore, there will be only mild effects of TANGO1 depletion on general protein secretion, more visible effect on overexpressed small proteins, and a severe impact on procollagen secretion. TANGO1 family proteins function at ERES to recruit a retrograde tether complex, which in turn recruits a specific subset of COPI-coated ERGIC membranes. The fusion of these membranes at the ERES results in the growth of an intermediate that does not form by simply pinching membrane from a donor compartment. Instead TANGO1 stabilises a transient pore or tunnel between the two compartments, through which bulky cargoes like collagen are transferred. The TANGO1 ring separates the ER from the ERGIC. This mechanism bypasses vesicle-mediated transport and is fundamentally different from how a COPI or COPII-coated vesicle is created. TANGO1 at an exit site creates an ‘isolation chamber’ across organelles including the ER, ERES and ERGIC, dedicated to cargo collection, sorting and quality control.

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