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Review

ER exit sites – Localization and control of COPII vesicle formation

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ABSTRACT

The first membrane trafficking step in the biosynthetic secretory pathway, the export of proteins and lipids from the endoplasmic reticulum (ER), is mediated by COPII-coated vesicles. In mammalian cells, COPII vesicle budding occurs at specialized sites on the ER, the so-called transitional ER (tER). Here, we discuss aspects of the formation and maintenance of these sites, the mechanisms by which cargo becomes segregated within them, and the propagation of ER exit sites (ERES) during cell division. All of these features are inherently linked to the formation, maintenance and function of the Golgi apparatus underlining the importance of ERES to Golgi function and more widely in terms of intracellular organization and cellular function.

1. Introduction

The endoplasmic reticulum (ER) is an interconnected network of tubules and cisternae throughout the cytoplasm (reviewed in [1]) and represents the entry point into the secretory pathway. After translation and translocation of secretory proteins into the ER lumen, rapid folding occurs and correctly folded proteins are transported towards the Golgi apparatus. The dynamic interplay between folding and export, termed proteostasis is itself a very exciting topic receiving considerable interest for its potential to intervene in a number of disease states [2]. Anterograde transport of correctly folded secretory cargo is mediated by the production of COPII-coated vesicles that bud from the ER [3]. In mammalian cells and the yeast *Pichia pastoris*, budding of the COPII-coated vesicles occurs at specific sites of the ER called transitional ER (tER) [4–6]. These vesicles subsequently fuse to generate, or fuse with pre-existing post-ER membranes of the ER–Golgi intermediate compartment [7]. We use the term ER exit site (ERES) to define the structures of the transitional ER and immediate post-ER compartments up to the ERGIC. This review focuses on the structure, organization and maintenance of these ERES.

2. Overview of COPII vesicle formation

We and others have recently reviewed the production of COPII-coated vesicles [8,9] and here, we try to discuss more specifically the spatial organization of COPII-dependent export. The first step in the assembly of the COPII coat is the activation of the small GTPase Sar1 through its guanine exchange factor Sec12. The GDP/GTP exchange leads to the exposure of an N-terminal amphipathic helix of Sar1, with which it can insert into the ER membrane [10,11]. This insertion causes membrane deformation and is ultimately required for membrane fission [11,12]. Through direct interaction with Sec23, Sar1 recruits the heterodimer Sec23–Sec24 [13]. The majority of cargo is captured through interaction with Sec24, which exhibits multiple independent cargo binding sites [14–16]. After the incorporation of cargo and the formation of the stable pre-budding complex, the outer layer of the coat is recruited to the ER membrane. This outer layer is composed of the heterotetramer Sec13–Sec31, which consists of two Sec13 and two Sec31 subunits [17]. Sec13–Sec31 can self-assemble into cage like structures with a cuboctahedral geometry [18]; the crystal structure reveals relatively weak inter-subunit interfaces and a geometry that could allow greater flexibility of the COPII coat, compared to other coats such as clathrin, in order to accommodate cargo of different shapes. The ordered assembly of these core components of the COPII coat are shown in Fig. 1.

Soon after budding, COPII vesicles uncoat due to the GTP hydrolysis by Sar1 [19]. The very slow intrinsic GTP hydrolysis rate of Sar1 is accelerated in two steps: first through the interaction with

Abbreviation: ERES, ER exit site

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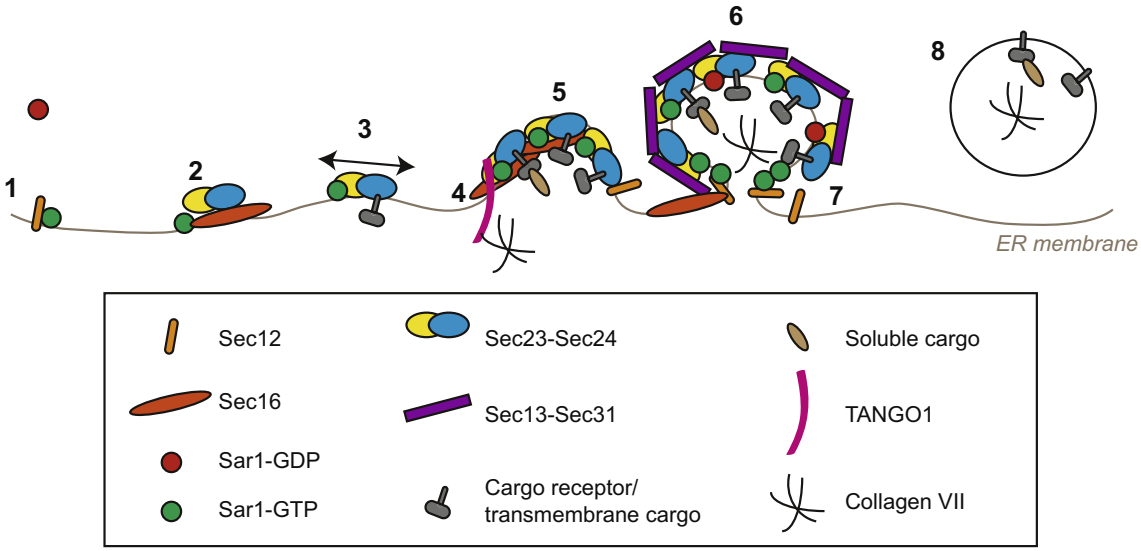

Fig. 1. Schematic of COPII vesicle formation. Step 1: Sec12-dependent exchange of GDP for GTP activates Sar1. Step 2: This directs recruitment of Sec23–Sec24 which then samples the ER membrane before (Step 3) engaging with transmembrane cargo. Sec16 could also play a role in coordinating clustering of such COPII–cargo interactions. Step 4: Propagation of the COPII coat is directed by coat–cargo interactions (including with soluble cargo via membrane receptors that are then included in the final vesicle as well as other adaptors such as TANGO1 that are ultimately excluded from the final structure). From experiments in yeast and human cells a role for Sec16 is again likely here. Step 5: Data suggest that this oligomeric structure including Sec23–Sec24 and significant cargo is sufficient to drive deformation of the membrane. Step 6: Recruitment of Sec13–Sec31 results in full assembly of the COPII coat and (Step 7) exclusion of proteins including Sec12 and TANGO1 (possibly through concerted GTPase activity on Sar1). Full coat assembly also drives vesicle scission (Step 8) through an unknown mechanism involving Sar1–GTPase activity.

Sec23 acting as GTPase-activating protein for Sar1 [13], and second through the binding of Sec13–Sec31 to the pre-budding complex, which increases Sec23-mediated GAP activity by an order of magnitude [20], presumably by optimising the interaction of Sec23 with Sar1 [21]. While inherent instability could present a problem with regard to stabilization of the COPII coat, the ongoing presence of Sec12 will provide a continuing supply of Sar1–GTP [22] and cargo–coat interactions are known to stabilize the pre-budding complex even in the presence of ongoing GTP hydrolysis by Sar1 [23]. This dynamic interplay between coat assembly and disassembly clearly has important implications for budding and we discuss these in more detail below; the reader is also referred to other reviews on this topic for a more in-depth discussion [24,25]. Sar1, Sec23–Sec24, and Sec13–Sec31 are the minimal machinery required to reconstitute COPII-dependent budding in vitro [26]; GTP-dependent budding requires Sec12 in addition [22]. In vivo, multiple other factors are likely to play key roles. Notably, COPII budding in mammalian cells is ATP-dependent and sensitive to protein kinase inhibitors [27,28]. This is also discussed in more detail below.

2.1. Cargo packaging: regulation of COPII assembly?

As stated above, the coupling of coat formation to cargo packaging is an intrinsic property of the COPII coat [29,30]. One of the best understood cargo receptors is the type I transmembrane protein ERGIC-53 [7,31]. This mannose-binding lectin is required for the export of several cargoes from the ER [31,32] as well as (along with other cargo receptors) for maintaining the structural integrity of the intermediate compartment [33]. Mutations in the ERGIC-53 gene cause autosomal recessive bleeding disorders resulting from the inability to package the blood clotting factors V and VIII [32]. ERGIC-53 interacts directly with Sec23 leading to the recruitment of ERGIC-53/cargo complexes into COPII vesicles [34]. After reaching the ERGIC, the cargo dissociates from the complex and ERGIC-53 is recycled back to the ER [35,36].

While one can easily envisage simple capture mechanisms operating for transmembrane cargo that can directly engage Sec24 [15,16], as well as soluble cargo that indirectly contacts the coat [37], complex issues arise for the packaging of large cargo molecules such as procollagen or lipoproteins [38]. Proteins such as Erv29p in yeast [37] and TANGO1 in mammals have been identified [39] that provide mechanisms to couple secretory cargo with the COPII coat. The transmembrane protein TANGO1 binds to collagen VII on the lumenal side of the ER membrane and can also bind to Sec23–Sec24 on the cytosolic side. Thus, it has the capacity to directly couple cargo packaging to coat formation which provides a mechanism to integrate coat assembly directly with cargo packaging [25]. Unlike other cargo receptors such as Erv29p, TANGO1 is not itself incorporated into the budding vesicle. TANGO1 is also implicated in the organization of ERES but it is unclear whether this is a specific role of TANGO1, or due to an accumulation of secretory cargo within the ER when its function is perturbed [39]. We do not intend to detail this here as it has been covered in other recent reviews [25,38].

Previously, the pre-budding complex of Sar1–Sec23–Sec24 and cargo has been considered insufficient to generate any deformation of the membrane, with curvature being induced on recruitment of Sec13–Sec31. Recent data suggest that this might not be the case. In the autosomal recessive human disorder cranio-lenticulo-sutural dysplasia, Sec23A is mutated [40] leading to inefficient coupling to Sec13–Sec31 [21,41]. Suppression of Sec13–Sec31 expression using RNA interference also leads to inefficient coupling of the inner and outer layers of the COPII coat [42]. In both cases the ER of cells is distended, consistent with a failure to export cargo (in both cases an apparent selective export of collagen), and is decorated with multiple budding profiles consistent with a defect in both constriction of the vesicle neck and also of fission. Notably though significant curvature is observed suggesting that accumulation of pre-budding complexes, presumably through concentration of Sar1–GTP [11,12] but perhaps also through a concentration of transmembrane domain-containing cargo, is sufficient to drive membrane curvature (Fig. 1). Clearly, further analysis of the mechanism of export of procollagen from the ER is going to provide key insight in to the control of the budding process and has obvious developmental and clinical relevance.

3. Definition and structure of ER exit sites

The organization of COPII dependent budding is very different between some of the key species studies. The existence of a stable population of ERES in *Saccharomyces cerevisiae* remains ill-defined. This could be due to smaller, more mobile ERES in this yeast compared to others such as *P. pastoris*. *P. pastoris* exhibits clear organization of COPII budding at specific ERES (see [43]). The pioneering work of George Palade and colleagues (see [4]) showed that, in mammalian cells, after their translation at the rough ER, secretory proteins enter specific sub-domains of the ER before being transported onwards to the Golgi complex. These sub-domains called transitional ER are regions of rough ER, which are devoid of ribosomes and show characteristic COPII positive buds [6]. The term ERES largely originates from light microscopy observations of the localization and dynamics of COPII proteins in cells. ERES encompass more than just the tER membrane itself, including the post-ER structures which likely represent COPII-coated tubulo-vesicular membranes prior to fusion with (or to become) the ERGIC. Mystery still surrounds the identity of the first post-ER membranes of the ERES. Evidence supports the existence of free vesicles that can be labelled with COPII proteins [44] with considerable tubular elements are also visible [5,45–47]. It is also unclear at what point these membranes become truly independent from the underlying ER membrane, resolution of this mechanistic detail will require a thorough analysis of the mechanism by which Sar1–GTP is able to mediate membrane fission [11]. This layout is described in more detail in [8]. In mammalian cells several hundred ERES can be found that are distributed throughout the cell cytoplasm and cluster in the juxtanuclear region [5] (Fig. 2A). ER exit sites, having an approximate diameter of ~0.5 μm, are relatively stable and immobile structures as determined in studies using time-lapse imaging [48,49]. The majority of ERES move only a short distance often coinciding with the movement of the underlying ER network itself and also of ERES themselves driven by kinesin-1 and dynein-1 [50]. ER exit sites are part of a larger structure referred to as the export complexes [5]. These complexes comprise of one or more bud bearing transitional ER elements facing towards a central cavity containing a number of vesicles and tubules, known as vesicular tubular cluster (VTCs) or ER–Golgi intermediate compartment (ERGIC) [51,52] (Fig. 2). Ultrastructural analysis and confocal microscopy studies revealed that VTCs are distinct from transitional ER, but interconnected with each other [5]. VTCs label with COPI [53,54], a second cytoplasmic coat complex. COPI is involved in retrograde transport from the ERGIC and Golgi back to the ER, transport towards the Golgi and also in intra-Golgi transport (reviewed in [55]).

3.1. The role of Sec16 in ERES organization

Over the past years evidence accumulated for a central role of the peripheral membrane protein Sec16 in the organization of ER exit sites. As with most other COPII components, Sec16 was first identified in the yeast *S. cerevisiae* in a screen for secretory pathway mutants [56]. Mutations in the SEC16 gene result in an accumulation of secretory pathway precursors within the cell [57]. Moreover, electron microscopy studies revealed a lack of 50 nm vesicles in these mutants [58]. Sec16p was determined to be a

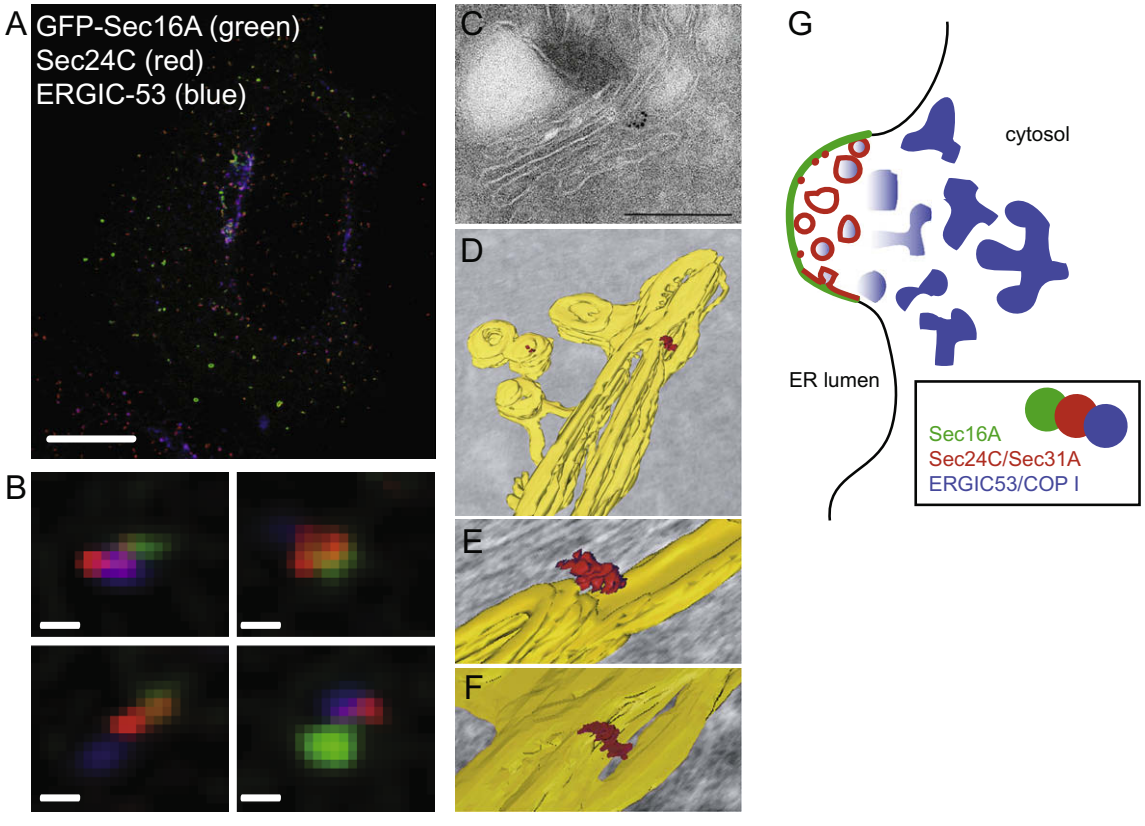

Fig. 2. Organization of ERES. (A) Images, and (B) enlargements, of cells expressing GFP-Sec16A that have been labelled with antibodies directed against Sec24C and ERGIC-53. Clear separation is seen between the three markers here using confocal microscopy and image deconvolution. (C) Immunogold labelling of ultrathin cryosections shows localization of Sec16A to cup-like structures in the vicinity of Golgi membranes. (D, with enlargements in E and F) shows electron tomography reconstruction so Sec16A-labelled membranes confirming the concave nature of Sec16A-labelled membrane. (G) Schematic model developed from the data in (A–F) and other published work. Sec16A is shown labelling tER membrane with other COPII components, Sec24–Sec24 and Sec13–Sec31 labelling the first post-ER membranes. These then coalesce to create the ERGIC to which COPI is recruited. This figure is compiled from data first published in Journal of Cell Science [67].

240 kDa peripheral membrane protein that is localized to the ER membrane [59]. Further research detected a direct interaction of Sec16p with the COPII components Sec23p, Sec24p and Sec31p [59–61]. Taken together these results strongly suggest a key involvement of Sec16p in the formation of COPII-coated vesicles. This hypothesis is strengthened by the results obtained by Supek and colleagues, who found that Sec16p binds to synthetic liposomes and facilitates the recruitment of Sec23p–Sec24p and Sec13p–Sec31p [62]. Moreover, Sec16p was found to stabilize the Sar1–GTP–Sec23p–Sec24p complex on liposomes possibly preventing premature disassembly after GTP hydrolysis. Sec16p could therefore act as a scaffold directing assembly of the COPII coat. The involvement of Sec16p in the organization of ER exit sites was further defined through work on the budding yeast *P. pastoris*. Discrete and clearly definable ERES in this species make this an excellent model organism to study the organization of early secretory pathway [43]. Through a genetic screen in *P. pastoris* a mutation called *dot1* was determined showing a general breakdown in tER organization; the *dot1* mutation was mapped to the SEC16 gene [63] and was shown to disrupt the steady-state localization of Sec16p to ERES.

Two mammalian orthologues of Sec16 have recently been identified – a longer Sec16A and a shorter Sec16B [64–66]. Most research has concentrated on Sec16A, which seems to be the primary orthologue having most similarity to Sec16 in other species. Sec16A is a 250 kDa protein showing a typical ER exit site localization [64–66]. First results confirming the involvement of mammalian Sec16A in ERES organization were obtained through overexpression and siRNA depletion studies. In both cases a disorganization of the ER exit sites was observed. In addition to that, depletion of Sec16A results in a delay and overexpression in an inhibition of ER to Golgi transport [64–66]. Sec16A interacts physically with the COPII subunits Sec23 and Sec13 [65–67]. Fluorescence recovery after photobleaching showed that Sec16A rapidly cycles on and off the membrane [64]. While there is a significant steady-state pool of Sec16A in the cytoplasm, recycling is slower than that of Sec23A and it has a greater immobile pool (around 50%) on the ER membrane [67]. This is consistent with the idea that Sec16A provides a platform for the COPII assembly.

We have recently used laser scanning confocal microscopy and electron microscopy to define the steady-state localization of human Sec16A in more detail [67]. Intriguingly, in more than 80% of cases Sec16A localizes to a region of the ER exit site spatially separated from Sec24C and Sec31A (Fig. 2B). This observation was confirmed by immunogold labelling of ultrathin cryosections with Sec16A and Sec31A, showing that in most cases the two proteins were between 100 nm and 600 nm apart. Only 14% of COPII structures showed colocalization of Sec16A and Sec31A [67], suggesting that assembly and budding events are rapid. These fast dynamics could explain this difference in steady-state localization despite clear interaction between these components. Moreover, electron microscopy (Fig. 2C) and 3D reconstructions from electron tomography (Fig. 2D) showed that Sec16A localizes to concave cup-shaped structures. It is not clear if curvature is required for ER exit site formation or whether Sec16A plays a role in generating these curved structures. The localization of Sec16A to ERES is independent from other COPII components [67] again consistent with a role as a bona fide organizer of COPII recruitment to the tER. Whether Sec16 has a critical role in COPII vesicle formation or acts as a regulator of this process remains to be defined. In *P. pastoris*, Sec16p is present at an order of magnitude lower abundance than other COPII components [63], consistent with the notion that it regulates COPII assembly.

Less is known about the function of the second mammalian orthologue Sec16B [65]. The 117 kDa protein is encoded by a gene previously described to express a regucalcin gene promoter region-related protein RGPR-p117 (see [68] and references therein). A GFP-tagged version of Sec16B was shown to localize to ERES ([65], A.B. and D.J.S. unpublished observations) and to be required for export from the ER [65]. Moreover, there is evidence that Sec16A and Sec16B function in a heteromeric complex as they were shown to co-immunoprecipitate [65]. However, work so far has been done on overexpressed Sec16B and endogenous protein could not be unequivocally detected using antibodies (A.B. and D.J.S., unpublished observations) although Sec16B mRNA can be found in all tissues examined [65]. It could therefore be possible that Sec16B expression is regulated on a post-transcriptional level and it is only required for certain cargo or at a certain cellular state. Future research is required to answer these questions.

The importance of Sec16 in ERES organization was further confirmed through the characterization of the *Drosophila* orthologue dSec16 [69]. dSec16 also localizes to ERES and in agreement with the findings for human Sec16A, depletion of dSec16 leads to a disruption of ERES and inhibits ER export. As shown for human Sec16A, dSec16 localizes to cup-shaped structures on the ER as determined by immuno-EM. Ivan and colleagues [69] revealed that dSec16 localizes upstream of other COPII subunits and most interestingly provided clear and convincing data that dSec16 directs recruitment of Sar1–GTP [69]. This contrasts with previous findings that suggested that Sec16 was recruited in a Sar1-dependent manner [64]. The explanation for this could be that Sar1–GTP is required to retain an ongoing active pool of Sec16 through prevention of its dissociation, rather than directly acting in its recruitment. This interpretation of the data would also agree with findings for *P. pastoris* Sec16p showing no change in distribution upon expression of Sar1–GDP [63].

From findings such as those described above, we develop a more precise picture of an ERES (Fig. 2D) in which we can define distinct membrane entities, at steady state at least. These are the transitional ER membrane itself, which we propose is defined by the presence of Sec16A [67], the nascent COPII-coated tubulovesicular compartment [6,44], and the first post-COPII membranes, which are likely to almost immediately become COPI-coated and therefore can most easily be defined as the ERGIC [7]. This of course considers the ERGIC as a stable compartment. There are currently two models explaining the role of VTCs in ER-to-Golgi transport (see [7]): in the first model, the transport complex model, the VTC evolves through fusion of COPII vesicles soon after their budding from the ER. The VTCs or transport complexes are then transported in a microtubule dependent manner towards the Golgi, where they fuse directly with the cis-Golgi or undergo homotypic fusion to produce a new cis-Golgi. The second model is the stable compartment model, which assumes that the ERGIC is a stable compartment with which COPII vesicles fuse. Cargo destined for the Golgi is delivered to the cis-Golgi in anterograde carriers requiring microtubules and the COPI coat. Evidence exists for both models; however the interested reader is referred to other reviews and primary research articles focussing especially on this topic [7,33,70].

The functional specialization of a sub-domain of the ER as transitional ER could have implications for cargo sorting. Borgese and colleagues have elegantly shown that transmembrane domain length dictates the selective partitioning of cargo in to ER export domains [71]. LRP6, a key protein in the canonical Wnt signalling pathway, is palmitoylated and it has been proposed that this serves to tilt the transmembrane domain to allow for efficient ER export [72]. Functional specialization of the tER and restriction of the site of COPII-dependent budding could act to spatially organize specific cargo receptors such as TANGO1 [39].

3.2. Correlation between ERES and Golgi structure

The number and spatial organization of ERES seems to some extent to be correlated with Golgi organization. This connection becomes clear when the arrangement of ERES in various organisms is analysed. The budding yeast *P. pastoris* shows a clear organization of ER exit sites with cells having an average number of four ERES [43]. The Golgi apparatus of *P. pastoris* is arranged as stacked Golgi cisternae and electron microscopy revealed that these cisternae are associated with ER exit sites [43]. A similar organization is found in the unicellular parasites *Trypanosoma brucei* and *Toxoplasma gondii*, which have one ER exit site adjacent to one stacked Golgi [73,74]. In mammalian cells several hundred ERES are distributed throughout the cytoplasm, abutting ERGIC elements; there is also an accumulation of ERES in the juxtanuclear region directly adjacent to a stacked Golgi apparatus. A juxtaposition of ERES and Golgi can also be detected in specialized cells like muscle fibres [75]. However, here the Golgi is not found as a compact unit close to the nucleus, but as individual elements dispersed throughout the fibre. In contrast, in *S. cerevisiae* the Golgi consists of individual scattered Golgi cisternae, which are not arranged in stacks [43]. An interesting relationship of ERES and Golgi is found in plant cells. Similar to the organization in most metazoa, plant ER exit sites are found in close proximity of small cisternal Golgi stacks [76,77]. However, current models propose that these two structures behave as a single secretory unit that is motile along the underlying ER [76]. The organization and dynamics of the ER/Golgi interface in plant cells show many similarities to that of mammalian cells but there are also many significant differences. Readers are referred to the literature for a more detailed discussion of this [78].

The reasons for a direct physical proximity between many ERES and Golgi membrane remain unclear. This could relate to more direct "fast" routes for secretory transport, i.e. directly coupling tER and Golgi units in mammalian cells would eliminate the need for long-range microtubule-based transport. Alternatively, this characteristic organization could be linked to either the biogenesis or morphogenesis of the Golgi itself (for example on exit from mitosis, see Section 3.4). Organisms such as *Drosophila* provide good evidence for this organization such as the differential function of tER/Golgi discrete units [79,80]. The reader is referred to the following review for an excellent discussion on this point and on the important distinction between biogenesis and morphogenesis of the Golgi [81].

3.3. Controlling ERES size

Considerable evidence points to a close correlation between ERES and Golgi structure. When ERES form de novo in *P. pastoris*, an adjacent Golgi structure forms at the same time [82]. Moreover fusion of two ER exit sites in *P. pastoris* was found to also lead to the fusion of the associated Golgi [82]. Similarly, formation of a new ER exit site during cell division in *Trypanosoma brucei* coincides with the duplication of the Golgi next to the new ERES [74]. Like mammalian ER exit sites, ERES of *P. pastoris* are long-lived, stable and were found to fuse upon collision [82]. When formed de novo, ERES are first observed as small fluorescent spots which then increase in size, reaching their full size within 10–15 min [82]. Cargo can directly influence COPII vesicle formation [83], providing a means to regulate the number and size of ERES when the secretory requirements of the cell change [84,85]. This observation is consistent with data obtained for mammalian cells, in which de novo formed ERES were found to recruit more YFP-Sec23A, possibly a mechanism to control size [86]. There needs to be a fine regulation between budding of COPII vesicles from ERES and the recruitment of new molecules to these sites in order to regulate their size [82]. Mathematical modelling [87], coupled with experimental work [88], suggests that this is achieved through a combination of the regulation of both ERES number and size.

3.4. ERES and Golgi dynamics in mitosis

Mammalian ER exit sites proliferate during interphase; cells in G2 show twice as many ERES as cells in G1 [49,86]. At the end of interphase, translocation of newly formed ERES towards the juxtanuclear region can be observed, which correlates with a relocation of a subset of sites and Golgi membranes above the nucleus. The distribution of ERES changes further throughout mitosis. During metaphase, the COPII components no longer associate with the membrane and are dispersed throughout the cytosol as defined by light microscopy [49,86,89,90], suggesting a disassembly of ER exit sites. This observation is consistent with data obtained by electron microscopy [91]. During mitosis, COPII components such as Sec23 and Sec13 become cytosolic [86,91] and there is a concomitant reduction in budding profiles, number and size of ERES during metaphase, proving their disassembly [89]. In the late metaphase up to telophase COPII components reassemble to spots as determined using Sec13-YFP [90]. Finally, during cytokinesis the cell exhibits the same number of ERES as in the early prophase [49].

During mitosis, the Golgi is known to breakdown (reviewed in [92]). First during prophase a fragmentation of the Golgi ribbon occurs, followed by the dispersion of the Golgi throughout the cytoplasm until telophase is reached. During telophase and cytokinesis Golgi fragments finally start to reappear concomitant with a reactivation of Arf GTPase recruitment [90,93]. This behaviour of the Golgi during mitosis coincides with that of ERES. The fragmentation of the Golgi takes place at the same time as the loss of Sec13 from ERES and the reassembly of the Golgi at the same time as that of the ER exit sites [49]. During prometaphase, Golgi fragments are found in close proximity of ERES; fluorescently labelled GalT marking the Golgi and fluorescently labelled Sec13 marking the ERES show a close co-alignment as determined by light microscopy [90]. This observation was confirmed by electron microscopy revealing tubulo-vesicular Golgi clusters in proximity of the ER. The reassembly of ERES and Golgi follow sequentially, reappearing telophase Golgi fragments seem to grow out of ER export sites after those have been reassembled themselves [90]. It is likely therefore that, while there is considerable evidence that the Golgi and ER remain distinct through mitosis [94], and that there are clear physiological reasons why one would wish this to be maintained [95], there is clearly a suppression of export from the ER at this time and the reformation of the full Golgi structure clearly coincides with a resumption of COPII-dependent export on exit from mitosis [90].

4. Additional factors controlling ERES function

Multiple proteins have been identified that impact on COPII function and/or ERES organization in cells. Many of these when overexpressed or depleted using RNA interference cause defects in COPII assembly, localization, or dynamics. The precise role of these factors remains unclear and it is highly likely that cargo- or tissue-specific functions are at the heart of this multiplicity. Such proteins include protein kinases such as PCTAIRE [96], and p38 MAP kinase [97], adaptors such as STAM [98], and potential regulators of other signalling pathways including p125 [99], and Nm23H2 [100]. Phosphatidylinositol-4-kinases have been implicated in COPII function [88,101]. The calcium binding protein ALG-2 has also been implicated in COPII assembly [102,103],

potentially providing a point of integration of calcium oscillations and ER export activity [104]. The dynactin subunit p150^{Glued} interacts with Sec23 [105], providing a direct link and integration with the microtubule cytoskeleton. Other factors such as the yeast protein Smy2p [106] have been identified in suppressor screens but precise functions are also unclear. A potential unifying theme here is that many of these proteins including Smy2p, p125, PCTAIRE, and the dynactin component p150^{Glued}, interact with the Sec23–Sec24 component of the coat. This would provide a potential link to the direct regulation of Sar1–GTPase activity since Sec23 acts as the GAP for Sar1. This has immediate implications for the potential control of coat assembly in the context of cargo packaging, a model already proposed for TANGO1 [25,39]. Protein phosphorylation is likely to play a key role here with many COPII components having been shown to be (e.g. Sec31 [107]), or are predicted to be (e.g. Sec16A) phosphorylated; indeed the same is true for COPI [108]. Clearly there are many features of the regulation of COPII-dependent budding from the ER that remain to be elucidated.

5. Conclusions

Since the discovery of the Golgi apparatus in 1898, our understanding of the mechanisms underlying its biogenesis and function has developed beyond expectation. Clearly it is important that we understand the interplay of this dynamic organelle with other components that feed in to its structure and function, especially the ER exit sites with which it has a unique relationship. The relevance of transport between the ER and Golgi to the generation and maintenance of healthy cells cannot be understated and, as has been beautifully illustrated in recent years, our understanding of the process of vesicle transport to and around the Golgi enables us to explain and potentially in the future alleviate, significant developmental and clinical disorders of membrane trafficking.

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