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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.

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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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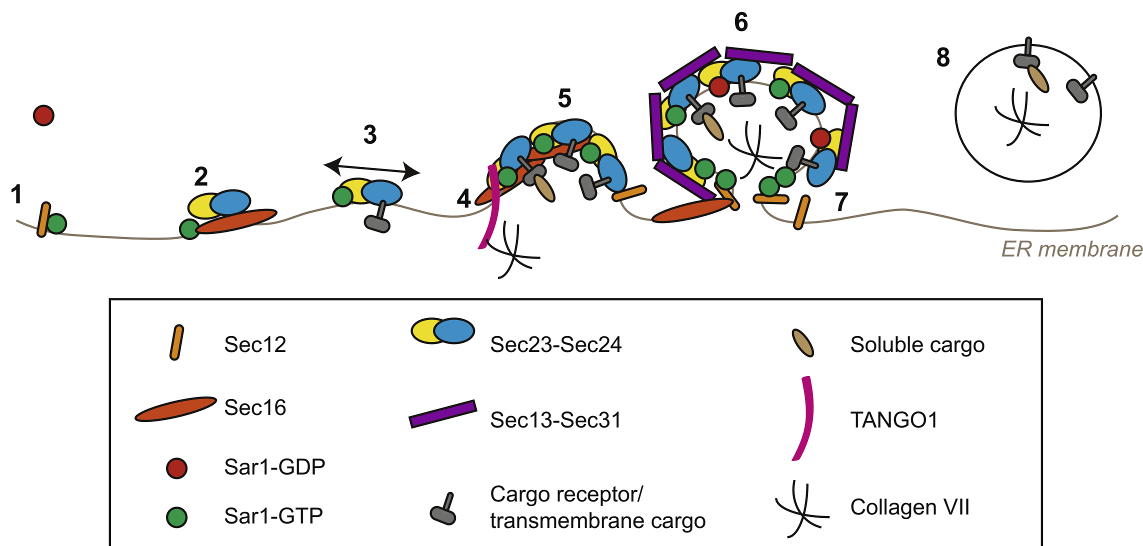

Fig. 1. Schematic of COPII vesicle formation. Step 1: Sec12-dependent exchange of GDP for GTP activates Sar1. Step 2: This directs recruitment of Sec23–Sec24 which then samples the ER membrane before (Step 3) engaging with transmembrane cargo. Sec16 could also play a role in coordinating clustering of 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.

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