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REVIEW

The small GTPase Sar1, control centre of COPII trafficking

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Sar1 is a small GTPase of the ARF family. Upon exchange of GDP for GTP, Sar1 associates with the endoplasmic reticulum (ER) membrane and recruits COPII components, orchestrating cargo concentration and membrane deformation. Many aspects of the role of Sar1 and regulation of its GTP cycle remain unclear, especially as complexity increases in higher organisms that secrete a wider range of cargoes. This review focusses on the regulation of GTP hydrolysis and its role in coat assembly, as well as the mechanism of Sar1-induced membrane deformation and scission. Finally, we highlight the additional specialisation in higher eukaryotes and the outstanding questions on how Sar1 functions are orchestrated.

Keywords: COPII; ER export; GTPase; membrane curvature; Sar1

Sar1 participates in the first step of the secretory pathway, the COPII-mediated export of newly synthesised proteins from the endoplasmic reticulum (ER) to the Golgi apparatus. Sar1 exists as a cytosolic pool in its inactive conformation, and associates with the ER membrane upon activation by Sec12, its cognate guanine-nucleotide exchange factor (GEF) (Fig. 1) [1]. Activated Sar1-GTP sequentially recruits Sec23-Sec24 and Sec13-Sec31 complexes to assemble the inner and outer layers of the COPII coat, respectively [2–4]. The COPII complex coordinates many different events in ER export, from cargo binding and concentration to deformation of the membrane into coated vesicular carriers (Fig. 1). Cargo selection is mediated by the Sar1-Sec23-Sec24 complex [5], primarily through cargo-binding sites on Sec24 [6,7], whilst membrane remodelling is orchestrated by a dynamic and complex interaction network involving all coat components [8–10]. GTP hydrolysis leads to the detachment of Sar1 and starts the uncoating process [2]. Whilst Sar1 itself has negligible GTP hydrolysis activity, this is significantly accelerated by the binding of Sec23 and Sec31, which act as GTPase-activating proteins (GAP) [4,11] (Fig. 1).

SAR1 is an essential gene, initially discovered as a multicopy suppressor for the temperature-sensitive mutation in *sec12* of *Saccharomyces cerevisiae* [12], the model organism for much of the Sar1 research. The *SAR1* gene codes for a 21-kDa protein, which belongs to the small GTPases superfamily, with conservation in the active site. Many small GTPases are lipidated to facilitate membrane association, for example, ADP-ribosylation factor 1 (Arf1), closely related to Sar1 and involved in intra-Golgi transport, is myristoylated at its N-terminus [13,14]. By contrast, Sar1 lacks any post-translational modifications [15], though it shares with other ARF family proteins an amphipathic N-terminal helix, which associates with membranes in a GTP-nucleotide and Mg^{++}-dependent manner [15]. Most ER membrane-associated Sar1 is found near the juxtanuclear Golgi ribbon in mammals [16,17]. Sar1 is further concentrated in so-called ER exit sites (ERES)

Abbreviations
ADE, area-difference-elasticity; AFM, atomic force microscopy; Arf1, ADP-ribosylation factor 1; CCD, central conserved domain; CLSD, cranio-lenticulo-sutural dysplasia; CMRD, chylomicron retention disease; CTR, C-terminal region; ER, endoplasmic reticulum; ERES, ER exit sites; ERGIC, ER-golgi intermediate compartment; FABP, fatty acid-binding protein; FIB-SEM, focussed ion-beam scanning electron microscopy; GAP, GTPase activating protein; GEF, guanine-nucleotide exchange factor; PRD, proline-rich domain; Sc, *Saccharomyces cerevisiae*; TFG, Turk-fused gene; TLC, thin-layer chromatography.

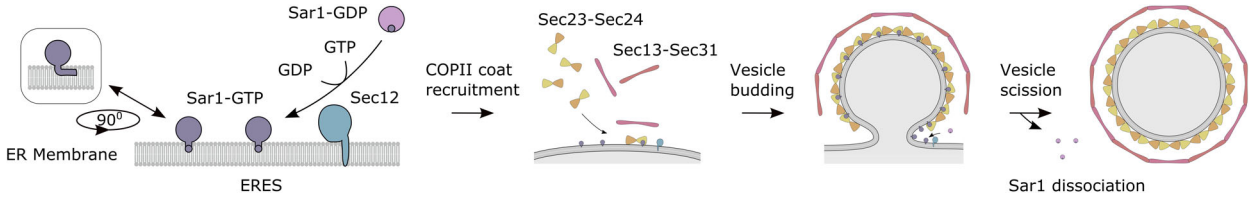


Fig. 1. Sar1 activation and COPII vesicle formation. Sar1 activation at ERES by its cognate GEF Sec12 results in membrane association (left, zoom on membrane). Multiple Sar1-binding events result in membrane deformation through sequential binding of Sec23-Sec24 inner and Sec13-Sec31 outer coat subunits. Continued accretion of coat components triggers GTP hydrolysis eventually leading to Sar1 dissociation and scission, followed by further uncoating and transport to the Golgi apparatus.

to facilitate secretion [18,19]. A cell has typically a discrete number of ERES, depending on the organism [20]. In vertebrates, the ERES are found in close contact with the ER-Golgi intermediate compartment (ERGIC), which functions as an acceptor site for the characteristic centralised Golgi ribbon [21].

The essential role of Sar1 in COPII-mediated membrane traffic has been extensively studied. *In vitro* assays using semi-intact/permeabilised cells [2,11,21,22] have been used to characterise many Sar1 mutations and their effects on COPII secretion (Table 1). Mutations blocking the GDP to GTP exchange or GTP hydrolysis both show defects in secretion, highlighting the need for GTP turnover [2,22]. From these studies, it is clear that Sar1 plays important roles in ER membrane deformation, cargo selection, vesicle formation, vesicle scission and uncoating, but the specific mechanisms and regulation remain debated. Here we review current knowledge on the small GTPase Sar1 with particular focus on three major aspects: (a) the role of Sar1 and of GTP hydrolysis in COPII assembly and disassembly; (b) Sar1 role in membrane remodelling, from curvature generation to scission; and (c) regulatory mechanisms that affect the processes above, and their increased complexity throughout evolution.

Role of Sar1 and GTP hydrolysis in coat assembly and disassembly

Activation of Sar1 at the ER exit sites

At ERES, Sar1 associates with the membrane upon GTP exchange induced by Sec12. Sec12 is an ER-resident integral type II membrane protein [1], which accelerates nucleotide loading on Sar1 by a billion times (Box 1 and Table 2, [32]), ensuring site-specificity of COPII budding. Next to its C-terminal membrane anchor, Sec12 features a cytosol-facing seven-bladed beta-propeller, which contains a conserved potassium-binding 'K-loop' crucial for GEF activity by wedging the Sar1 nucleotide-binding site open to facilitate exchange [42,43].

Structure of Sar1 and interactions with the COPII coat

Sar1 contains a nucleotide-binding pocket canonical to small GTPases and has high structural homology with other members of the ARF family (Fig. 2A) [28,49]. Two switch regions (Switch I (48–59) and Switch II (78–94) for hamster Sar1A2) change conformation upon nucleotide binding and hydrolysis. The nucleotide-binding site is partly formed by a conserved GxxxxGKT[39] ('G-1') motif and incorporates a magnesium atom. Upon guanine-nucleotide exchange, Sar1 undergoes conformational change leading to two main events: the amphipathic N-terminal helix is exposed by displacement of the ß2-ß3 hairpin mediating membrane binding [8,9,29], and its affinity for the inner coat increases leading to an extensive interaction interface with Sec23 encompassing both switch I and II regions and the ß2-ß3 hairpin ([49], Fig. 2B). Finally, metazoan Sar1 features a unique structural element, the extended omega loop (156–171 for hamster Sar1A2, Fig. 2A), which was shown to be indispensable for Sar1 self-association [33], a role also attributed to the apical helix [50]. Self-association of Sar1 might be important in the membrane deformation mechanisms discussed below.

GAP activity of the COPII coat is crucial for vesicle formation

In order to achieve productive COPII budding, coordinated assembly and release of the inner and outer coat complexes are critical. Membrane-associated Sar1 interacts with Sec23-Sec24 and Sec13-Sec31 in a sequential fashion, that is, the outer coat cannot bind Sar1 by itself [4]. Higher order associations within the coat are orchestrated by a complex network of interactions between and across inner and outer coat

Table 1. Mutations of Sar1 and associated phenotypes. List of Sar1 mutations correlated with correspondent biochemical effects. Obvious misfolding mutations, as well as frameshift or deletion variants were not included. Organisms are abbreviated as *Ce*, *Caenorhabditis elegans*; *Cg*, *Cricetulus griseus*; *Hs*, *Homo Sapiens*; *Pp*, *Pichia pastoris*; *Sc*, *Saccharomyces cerevisiae*. –, Not reported; AD, Anderson's disease; CMRD, chylomicron retention disease; ts, temperature sensitive.

Mutation – effect	Organism	Membrane interaction defect	Secretion defect	Growth defect	Literature
Nucleotide binding					
E112K	*Sc*	No binding	Moderate	ts	[11,23]
G37R	*Hs* Sar1B	No binding[a]	Large cargo	AD	[24]
D137N	*Hs* Sar1B	Impaired binding[a]	Large cargo	CMRD	[24]
S179R	*Hs* Sar1B	No binding[a]	Large cargo	CMRD	[24]
S197I – L181P	*Hs* Sar1B	No binding[a]	Large cargo	AD/CMRD	[24]
Nucleotide exchange – GDP lock					
D32G	*Sc*	No binding	Moderate	ts	[11,23,25]
T34N	*Pp*	No binding	Strong	Strong	[26,27]
T39N	*Cg*	No binding	Strong	–	[17,21,28–30]
T54A	Sc	No binding	Strong	Strong	[11,25]
GTP hydrolysis – GAP irresponsive					
H77L	*Sc*	Vesicles formed *in vitro*[b]	Strong	Strong	[11,25]
H75G	*Ce*	No remodelling on artificial membranes	–	–	[31]
H79G	*Cg* Sar1A2	beads-on-string morphology—no scission	Strong	–	[21,28–30]
Membrane binding					
Δ23 – Sar1Δ [1-23]	*Sc*	No binding	Strong	–	[8]
Δ9 – Sar1Δ [2-9]	*Cg* Sar1A2	No binding	Strong	–	[28,29]
Δ9 – Sar1Δ [1-9]	*Sc*	No binding	Strong	–	[28]
Δ6 – Sar1Δ [1-6]	*Sc*	Quasi-normal binding	None	–	[28]
Δ4 – Sar1Δ [1-4]	*Cg* Sar1A2	Quasi-normal binding	None	–	[28]
W4A | IF[6-7]AA | W9A | F10A	*Sc*	Impaired binding—impaired scission (W4A)	Moderate	–	[8]
D5A | RD[11-12]AA	*Sc*	Quasi-normal binding	None	–	[8]
F3A | F3D | F5A	*Cg* Sar1A2	Quasi-normal binding	None	–	[28]
F5D | FIF[345]AAA	*Cg* Sar1A2	No binding	Strong	–	[28]
FPF – Sar1[Y9F G11P S14F]	*Cg* Sar1A2	Quasi-normal binding—impaired scission	Moderate	–	[29]
Self-oligomerisation					
T158A	*Cg* Sar1A2	Normal	Strong	–	[28]
QTTG[156-159]AAAA	*Cg* Sar1A2	Normal	Large cargo	–	[33]
Other/unknown					
N132I	*Sc*	Unknown	Moderate	ts	[23,25]
N134I	*Cg* Sar1A2	Unknown	Moderate	–	[17]
G187fsX199[c]	*Hs* Sar1B	Unknown	Large cargo	CMRD	[24]
K36M	*Sc*	Unknown	Strong	Strong	[25]
D73V	*Sc*	Unknown	Strong	Strong	[25]

[a]Predicted effect on activity, no direct experimental evidence.; [b]Vesicles are observed with these mutants but are thought to occur due to trituration based on mammalian work.; [c]The mutated allele replaces amino acids 187–198 of Sar1B with the amino acid sequence LRRRLPLDGTVH.

subunits. Lateral assembly between Sec23-Sec24 inner coat subunits is in turn reinforced through interactions with the outer coat protein Sec31, whose triple-proline motifs in the proline-rich domain (PRD) bind across neighbouring Sec23 molecules ([9,10], Fig. 2B,C). Importantly, coat assembly is inherently coupled with disassembly as Sec23 acts as a GAP for Sar1, accelerating GTPase activity 9–15 fold [11,51] and leading to Sar1 release from the membrane [2]. Sec23 stimulates GAP activity both by stabilising productive interactions of GTP with switch I and by inserting an arginine finger (R722 in *Saccharomyces cerevisiae* (*Sc*) Sec23) in the active site, coordinating GTP phosphates and rendering them more susceptible to nucleophilic attack [49]. Binding of the outer coat further accelerates Sec23 GAP activity 2–10 fold (Box 1 and Table 2, [4,39,44,45]). This acceleration can be mostly attributed to a fragment of 35–40 amino acids in the

Box 1. Experimental methods to measure Sar1-GTP dynamics.

Early studies [11,15] used radioactively labelled GTP (e.g. [^{32}P]GTP) to chart nucleotide exchange and hydrolysis (combined with thin-layer chromatography (TLC) to directly assess hydrolysed GTP [34,35]). More accessible and user-friendly approaches have been benchmarked against this method. In a seminal paper, Antonny et al. [4] compared radioactive assays with a dynamic light scattering of COPII binding and followed the tryptophan fluorescence of Sar1 in these reactions. Due to tryptophan in the switch II region (W84 in *S. cerevisiae* Sar1, conserved in other G-proteins and Arf1 [36–38]), Sar1 has a higher fluorescence emission at 340 nm when bound to GTP than GDP [4]. With this assay, controls using nonhydrolysable analogues are warranted to exclude changes in fluorescence that are due to factors other than GTP hydrolysis (e.g. addition of other components). These controls have been sparse in the wide literature since their original implementation. Based on our own experience, binding of COPII proteins to Sar1-GTPγS can also cause a decrease in tryptophan fluorescence of Sar1. As an alternative, fluorescent MANT-GTP can be used [39], although fluorescence is mostly dependent on the local environment of the GTP product rather than the nucleotide state, and GTP hydrolysis kinetics of the modified GTP may be altered [40]. Finally, to overcome the shortcomings of measuring the nucleotide state indirectly, a commercial end-point assay was developed (GTPase-GLO™, Promega) converting unreacted GTP to a bioluminescent product in a two-step assay, improving sensitivity over classical chromogenic malachite green assays [41]. This assay was used to follow concentration-dependent Sar1-GTP hydrolysis [31], though has not yet found widespread use.

It remains unclear how GTP hydrolysis rates may be influenced by different methodologies or inconsistent activities of purified protein batches, as significant discrepancies have been reported in the literature (Table 2). In conclusion, whilst using tryptophan or MANT-GTP fluorescence offers the opportunity to follow GTP kinetics in real time, direct assessment of actual hydrolysis should be considered as a control, especially when studying the effect of other COPII proteins on Sar1 kinetics. It is noteworthy that methods measuring nucleotide hydrolysis directly in real time have been extensively used elsewhere (e.g. [46–48]) and these could find implementations in the future especially in complex setups.

Table 2. Experimental rates of Sar1-GTP turnover. Only including reported experimental rates, not qualitative assessments mostly made using the tryptophan fluorescence method. Experiments from the same study are indicated with '&, ¥, £, $, €'. Note the more pronounced effect for yeast outer coat (£) binding compared with the mammalian counterpart ($, €). *S. cerevisiae*: *Saccharomyces cerevisiae*. FA, Filter Assay; TLC, thin-layer chromatography; Trp, tryptophan.

GTP exchange		On rate (s^{-1}·Mol^{-1})	Organism	Method	Literatures
Sar1		1.5×10^{-3}	*S. cerevisiae*	Radioactive FA	[15]
	&	5×10^{-3}	*S. cerevisiae*	Trp-fluorescence	[32]
Sar1-Sec12	&	1.5×10^{6}	*S. cerevisiae*	Trp-fluorescence	[32]
		9.5×10^{6}	*S. cerevisiae*	Trp-fluorescence	[42]
		10×10^{6}	*S. cerevisiae*	Trp-fluorescence	[43]
GTP hydrolysis		**Hydrolysis (s^{-1})**	**Organism**	**Method**	**Literatures**
Sar1		1.8×10^{-5}	*S. cerevisiae*	Radioactive TLC	[15]
	¥	5.8×10^{-5}	*S. cerevisiae*	Radioactive TLC	[11]
Sar1-Sec23-Sec24	¥	5.2×10^{-4}[a]	*S. cerevisiae*	Radioactive TLC	[11]
	£	1.5×10^{-2}	*S. cerevisiae*	Mant-GTP fluorescence	[39]
	$	8.5×10^{-4}	Human Sar1A	Trp-fluorescence	[44]
	$	8.4×10^{-4}	Human Sar1B	Trp-fluorescence	[44]
	€	2.4×10^{-3}	Human Sar1A	Trp-fluorescence	[45]
	€	1.6×10^{-3}	Human Sar1B	Trp-fluorescence	[45]
Sar1-Sec23-Sec24-Sec13-Sec31	£	$6.6–10 \times 10^{-2}$	*S. cerevisiae*	Mant-GTP fluorescence	[39]
	$	4.5×10^{-3}	Human Sar1A	Trp-fluorescence	[44]
	$	3.0×10^{-3}	Human Sar1B	Trp-fluorescence	[44]
	€	4.7×10^{-3}	Human Sar1A	Trp-fluorescence	[45]
	€	3.2×10^{-3}	Human Sar1B	Trp-fluorescence	[45]

[a]Calculated based on reported acceleration effect (8.9×) in the same publication.

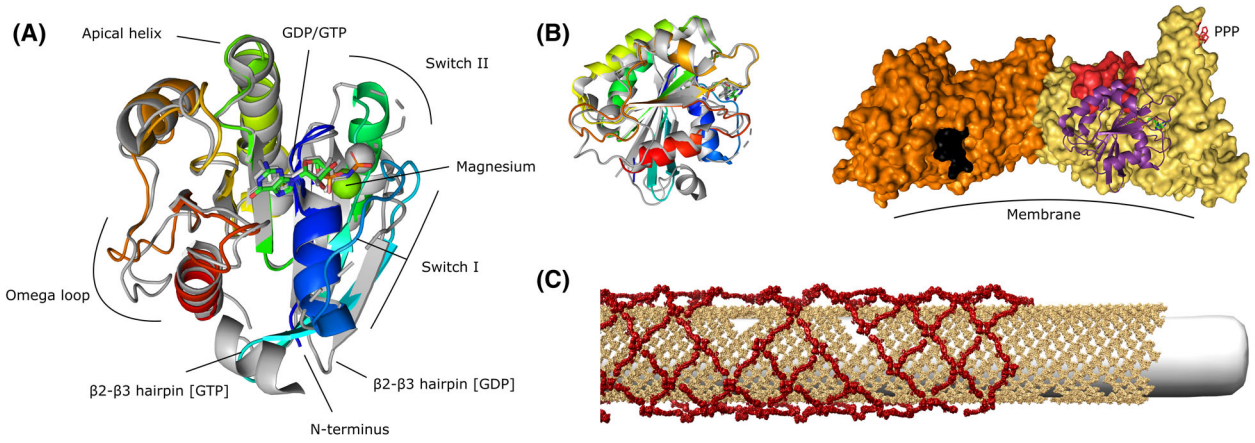


Fig. 2. Structure of Sar1 and interactions with COPII coat. (A) Atomic model of *Saccharomyces cerevisiae* Sar1 bound to GMP-PNP (non-hydrolysable GTP analogue) in rainbow representation (blue-red, N-C-terminus, PDB: 6ZGA). Superimposed in grey is *Cricetulus griseus* Sar1A2 complexed with GDP (PDB: 1F6B). The switch regions of *C. griseus* Sar1A2 (dashed lines) and extended omega loop are highly flexible. Comparison reveals the displacement of the β2-β3 hairpin in response to the nucleotide state, with the hairpin closing off the cavity for N-terminal helix binding in the GTP state. (B) Left: 90-degree rotated view of Sar1 as in (A) corresponding to its orientation in the Sar1-Sec23-Sec24 inner coat complex (right). Sar1 in purple, Sec23 in yellow and Sec24 in orange surface representation. Sec31 activating fragment (superimposed from PDB: 2QTV) is mapped in red, with triple-proline motive (PPP) positioned to bridge inner coat lattice subunits. One of the cargo-binding sites on Sec24 is depicted in black (cargo peptide in 'B'-site from PDB: 1PD1). (C) Reconstruction of an *in vitro* assembled tubule from subtomogram averaging [10]. Outer coat subunits (Sec13-Sec31, red) roughly span 2 inner coat lattice subunits (orange) but are flexibly tethered.

intrinsically disordered PRD of Sec31 (residues 907–943 in *Sc*Sec31). A crystal structure of Sar1/Sec23 co-crystallised with Sec31 active fragment shows that most of the involved Sec31 residues (920–942 in *Sc*Sec31) bind to Sec23, but the region between 907 and 922 binds to Sar1 [39]. Acceleration of GAP activity is caused by the interaction of W922 and N923 with the catalytic H77 residue in the *Sc*Sar1 switch II region, positioning it so that it primes the coordinated water molecule for the nucleophilic attack [39].

Sec16 as a conserved modulator of GTPase activity

Sec16 is an essential gene for ER-to-Golgi secretion [52]. It codes for a large and highly disordered protein, localising to ERES [26,53–56], capable of binding all of the COPII components through two conserved domains (Central conserved domain (CCD) and C-terminal region (CTR)) and a functionally conserved region in the N-terminal part [27,52,55–61]. Sec16 promotes COPII assembly on membranes by acting as a scaffolding factor [59]; in addition, it has been shown to negatively regulate Sar1-GTP hydrolysis. This effect has been attributed either to the binding of Sec16 CTR to Sec23 [56] or to the interaction of the CCD with Sec24, which could couple cargo loading to GTP hydrolysis control [61]. In both cases, the interaction of Sec16 with the Sec23-Sec24 complex precludes recruitment of the outer coat, thus inhibiting Sec31-mediated acceleration of GTP hydrolysis. Notably, Sec16 stays associated with the ER membrane and is not found in coated vesicles [55], possibly stabilising Sar1-GTP at the base of COPII buds.

Sar1-GTP cycles mediate cargo concentration in directional transport

Whilst it is well-established that some components of the COPII coat stimulate GTP hydrolysis by Sar1, uncoating as a result of this is still poorly understood. Other factors might stabilise the coat after GTP hydrolysis, resulting in an apparent uncoupling of GTP hydrolysis and uncoating. For instance, the presence of cargo can increase the number of Sec12-mediated Sar1-GTP-loading cycles occurring without the disassembly of the inner and outer coats [62,63]. These data are supported by FRAP experiments of ERES in cells, where Sar1 is less long-lived compared with Sec23-Sec24 in a cargo-load-dependent manner [64]. In this scenario, coat subunits are kept in place through cargo-Sec24 interactions whilst Sar1 is actively being exchanged, and this has been proposed to facilitate the concentration of cargo in vesicles. Sar1-Sec23-Sec24 complexes can actively discern cargo from non-cargo in a delicately tuned mechanism, dubbed the

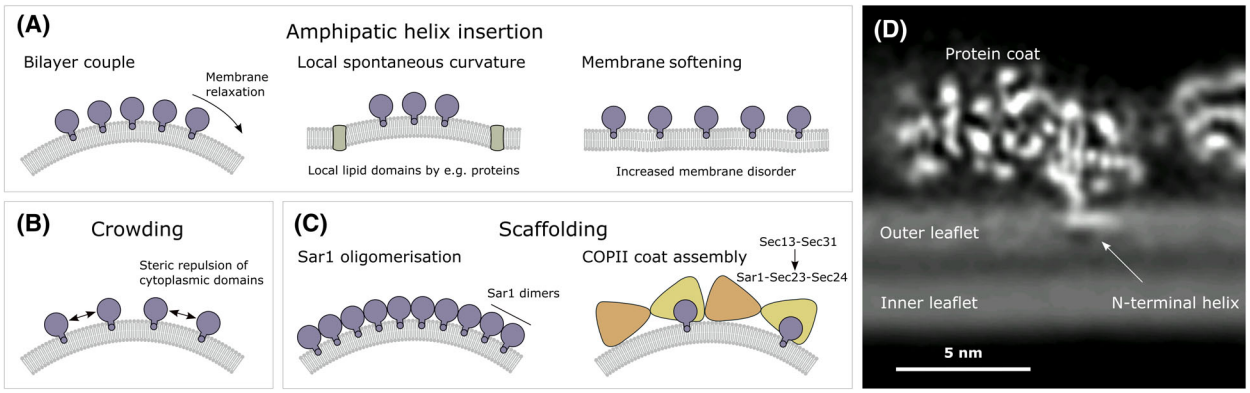


Fig. 3. Mechanisms of curvature induction by Sar1. Sar1-GTP (purple) inserts into the membrane and deforms it according to three main mechanisms. (A) Insertion of the amphipathic helix introduces changes in the membrane properties causing it to curve according to a bilayer-couple global model or a local spontaneous curvature model, which can be combined into the ADE model. Alternatively, amphipathic helix insertion can cause membranes to become more readily deformable (softening). (B) Repulsion of Sar1 molecules to compensate for unfavourable protein clashes leads to membrane curvature due to the difference in pressure between inner and outer leaflets (membrane crowding). (C) Membrane curvature obtained through protein scaffolding (Sar1 oligomerisation, COPII coat binding). Sequential Sec23-Sec24 and Sec13-Sec31 binding events reinforce COPII lattice formation. (D) Slice through a side view of a subtomogram averaging map of membrane-assembled COPII coat [9]. Electron density is white on black background, with N-terminal helix showing a kink and penetrating only the outer leaflet of the membrane.

kinetic proofreading model [63]. *In vivo*, this appears dependent on cargo crowding, as the active recruitment of cognate cargo precludes ER-resident proteins to enter vesicles [65,66]. Note that both Sar1-dependent and -independent cargo exist, suggesting that different avenues of cargo concentration in secretory membranes exist [67]. On vesicles, the coat is thought to be maintained even after Sar1 has dissociated [2] and at least for yeast, only disassembles upon phosphorylation by the Hrr25p kinase prior to cargo delivery at the Golgi [68]. In metazoa, Turk-fused gene (TFG) is found to promote uncoating by outcompeting Sec31 for Sec23 post-budding [69]. TFG is dependent on Ca^{++} and forms oligomeric assemblies stabilising the ER-ERGIC interface [70,71].

Sar1-membrane interaction and role in remodelling

Mechanisms of membrane deformation by Sar1

In order to form COPII vesicles, the membrane at ERES needs to be actively remodelled and Sar1 binding is believed to be one of the drivers of increased membrane curvature. A number of mechanisms for Sar1-mediated membrane deformation have been proposed, which can be grouped into three major modalities: amphipathic helix insertion, membrane crowding and scaffolding. Each of these modalities is supported by experimental and simulated data in ways that can be complementary but are sometimes difficult to reconcile. In this section, we attempt to summarise the proposed models (Fig. 3A–C).

Amphipathic helix insertion

Upon GTP-loading by Sec12, a conformational change exposes the N-terminal 20 residues of Sar1. These form an amphipathic helix that is stabilised by insertion in the membrane [8,29]. Deletion of the helix abolishes interaction with the membrane, whilst increasing [29] or decreasing [8,28] the hydrophobic character of the helix leads to a decrease in vesicle budding efficiency in part due to reduced membrane binding. A conserved STAR motif, consisting of bulky hydrophobic residues, seems especially important for Sar1 activation and membrane association [28]. Conversely, fusing the helix to a nonrelated protein (e.g. GST) can result in binding of the fusion protein to membranes and their destabilisation, yet without formation of tubes or vesicles [29]. Direct evidence of Sar1 helix insertion comes from cryo-electron tomography studies of reconstituted COPII-coated tubules [9]. High-resolution subtomogram averaging of the membrane-assembled coat revealed that Sar1 inserts its kinked N-terminal helix into the membrane outer leaflet to a depth of about 10–12 Å (Fig. 3D), confirming earlier models based on its amphipathic nature [8]. Together with its shallow insertion, that is just below the lipid head groups, the large number of hydrophobic residues increases the 'footprint' of

the helix leading to distortion of the head groups of the lipids, pushing open the outer leaflet of the membrane [8].

But how does the insertion of a helix induce curvature? In the 'bilayer-couple' model [72], large numbers of inserted helices that only penetrate the outer leaflet create a global area difference between the leaflets leading to positive curvature to compensate for the area asymmetry, relaxing the elastic energy over the membrane system (Fig. 3A, left panel). Alternatively, individual insertions of the amphipathic helices shape membranes through a local spontaneous curvature mechanism (Fig. 3A, middle panel), where the global effect is reduced to a simple local wedge mechanism comparable to the one created by lysophospholipids [73].

Local and global effects of the amphipathic helix insertion mechanism are brought together in the area-difference-elasticity (ADE) model. Here, the local membrane bending energy and the elastic stretching energy of the individual monolayers are minimised [74]. Interestingly, the ADE model predicts that an outer monolayer expansion as low as 0.1% can be enough to induce curvature in small lipid systems, such as GUVs [74,75]. This model predicts that the induced stress should result in the formation of beads-on-a-string [74], which are indeed experimentally observed when Sar1 is kept in an active state [33,76,77], also reminiscent of the deformations induced with nonhydrolysable GTP analogues in permeabilised cells [21].

However, considering the elastic energy of larger membranes, it is unlikely that curvature can be explained solely by area asymmetry induced by amphipathic helix insertion [73]. Based on computational models [78], Stachowiak et al. [79] posit that the inserted mass required to induce curvature should reach > 10% of the membrane surface for highly curved membranes (tubes of only 35 nm diameter). By contrast, the ENTH domain of epsin, a similar sized GTPase to Sar1, can efficiently induce membrane curvature at 20–50% surface coverage, with an inserted mass footprint as low as 2% [79]. When factoring in the size of the Sar1 cytoplasmic domain as part of the Sar1-Sec23-Sec24 heterotrimer [9], the Sar1 helix would occupy only 1–2% of the membrane area, even assuming 100% coverage. A study [77] has shown hamster Sar1 alone at concentrations > 7 μM can generate tubules, which are coated with Sar1 dimers. Whilst it is unknown whether Sar1 polymerisation is relevant *in vivo*, this mechanism would lead to helix insertion of up to 25% of the membrane area, satisfying the requirements for membrane deformation [77]. Interestingly, for the similar Arf1 GTPase of the COPI system, local concentrations on the membrane equivalent to 2 mM have been proposed [80], indicating this is at least a possibility.

At lower concentrations, thought to be more representative of typical Sar1 steady-state levels, it has been suggested that Sar1 merely softens the membrane (Fig. 3A, right panel), rather than imposing curvature itself [81,82]. This would make membranes more readily deformable through the different mechanisms described below.

Membrane crowding

Stachowiak et al. [79] proposed curvature is driven by 'membrane crowding' where the increasing lateral pressure created by colliding cytosolic domains of Sar1 at high concentrations is released by increasing membrane surface upon curvature (Fig. 3B). This effect can be mimicked by artificially associating the Sar1 N-terminal deletion mutant (Δ23, Table 1) to membranes (e.g. through his-tag binding to Ni-NTA lipids), as well as by binding of unrelated proteins (e.g. GFP) [79]. Whilst the amount of Sar1 diffusing on the ER membrane is unknown, crowding might make a relevant contribution to membrane deformation if local concentrations at ERES are sufficiently high.

Scaffolding

At high concentrations, mammalian Sar1 has been proposed to form regular arrays, with long-range Sar1 interactions not necessarily confined to dimers, which were posited to induce curvature via scaffolding (Fig. 3C, left panel) [33,77]. However, Sar1 scaffolding action is thought to be delivered mainly as a member of the assembled COPII coat.

The association of the COPII coat to membranes is Sar1-dependent and is enhanced by the presence of acidic and lysophospholipids [3,83]. Similar to proteins of the Bar family [84], the inner coat complex Sec23-Sec24 can act as a scaffold and aid membrane bending through its intrinsic curvature (Fig. 3C, right panel). X-ray crystallographic studies of the Sec23-Sec24 heterodimer (Fig. 2B) revealed a biconcave structure enriched in basic residues whose shape corresponds to the curvature of a canonical COPII vesicle [49,85].

The outer coat formed by Sec13-Sec31 is thought to also contribute to membrane deformation, as the rod-shaped Sec13-Sec31 heterotetramers self-assemble into polyhedral cages through interactions of their N-terminal β-propeller domains to form vertices [86]. These cages can exhibit different curvatures because of flexibility at hinges along the rods and of variations of

angles at the vertices [87]. Interestingly, Sec31 lacking the N-terminal β-propeller domains can support the formation of COPII-coated tubules from GUVs *in vitro*, indicating that inner coat assembly drives tubular curvature without the need for Sec13-Sec31 cages [10]. However, when the inner coat lattice interactions are weakened, the presence of the Sec31 β-propeller domain causes a switch from tubular to spherical curvature, indicating that both layers contribute to determining the overall membrane shape [10].

Moreover, in *in vitro* systems, the COPII coat can induce spherical structures when Sar1 lacks its N-terminal helix (Δ23, Table 1) and is artificially associated with the membrane, in a Sec13-Sec31-dependent manner [8]. Collectively, these data point to a model where Sar1 by itself can drive membrane deformation via amphipathic helix insertion and molecular crowding, but interaction with other coat proteins and the multiple interactions between coat subunits are needed to determine the various curvatures seen in cargo-transporting vesicles.

Vesicle scission and its relation to GTP hydrolysis

Once a coated membrane bud is formed, it needs to detach from the ER to become a vesicle and transport cargo towards the Golgi. Early observations reported the formation of small spherical vesicles in the presence of nonhydrolysable guanine-nucleotide analogues such as GTPγS and GMP-PNP, suggesting GTP hydrolysis was not needed for membrane scission [2,3,8,15,22,88,89]. However, it was later realised that this was most likely an artefact of the experimental procedures involving centrifugation steps. Using permeabilised cells [21,29] and in *in vitro* systems with minimal trituration [9,10,76,90], tubules or beads-on-a-string were obtained, indicating that COPII can bind and deform membranes, but the scission of vesicles does not occur in the absence of GTP hydrolysis. Sar1 point mutants altering the amphipathic character (Table 1) of the N-terminal helix show reduced scission, indicating the importance of the helix in this step of membrane remodelling [8,29]. Using AFM, Sar1 was shown to remodel membranes efficiently only in the presence of GTP [31]. The question thus arises how scission is orchestrated and why GTP hydrolysis would be required. A number of models have been proposed, which we briefly summarise below.

In one model, Sec12-mediated GTP exchange and COPII-induced GTP hydrolysis would maintain a high density of dynamically cycling Sar1 at the base of the bud, leading to destabilisation of the bilayer to promote scission. Sar1 has a higher affinity for membranes of increasing curvature [31], which further concentrates Sar1 at regions of high curvature such as the neck of a budding vesicle.

Because Sar1 can self-oligomerise to form narrow tubules [33,77], it has also been proposed that an increase in self-interactions might be responsible for Sar1 concentration localised at the base of the budding vesicle, progressively constricting the neck through continued GTP-loading and hydrolysis cycles, in a manner not dissimilar to dynamin [33,91,92]. Alternatively, Hariri et al. posited that Sar1 preferentially self-associates on low-curvature membranes and propagation of the Sar1 lattice at the base of the bud could lead to scission [77]. In the latter model, the timing of bud formation and cargo loading would still be dependent on GTP turnover, but scission itself would not. Finally, the local high concentrations of Sar1 at the neck might drive vesicle scission through crowding where steric repulsion of the cytoplasmic domains increases curvature to the extent that scission becomes favourable [93].

Lipid composition might also contribute significantly to Sar1 role in scission. Sar1 prefers membranes with distinct rigidity characteristics, localising to the disordered phase [33,83]. At the same time, certain lipid compositions leading to more ordered phases are shown to be more prone to stress and packing rearrangements leading to scission [74,94]. This perceived contradiction could be explained if Sar1 itself, possibly together with other COPII proteins, induces the raft-like ordered lipid phase needed for scission at the neck of a budding vesicle, but this remains to be investigated. Furthermore, Sar1 can also activate lipid-modifying enzymes (e.g. phospholipase D) that might change local lipid composition [95], and blocking lipid turnover inhibits COPII vesicle formation [96]. However, data on the interactions between Sar1 and specific lipids, and its importance in remodelling are currently incomplete and hard to interpret.

Regulation of Sar1 across species

Evolutionary specialisation of the Sar1-GTPase

The increased complexity in organisms throughout evolution brings additional challenges in cargo secretion. The higher demand to regulate secretion is reflected in the increased number of paralogues of the COPII proteins and in the presence of additional modulatory factors (Fig. 4). Yeast [12], *C. elegans* [31] and *D. melanogaster* have a single Sar1 gene, but most

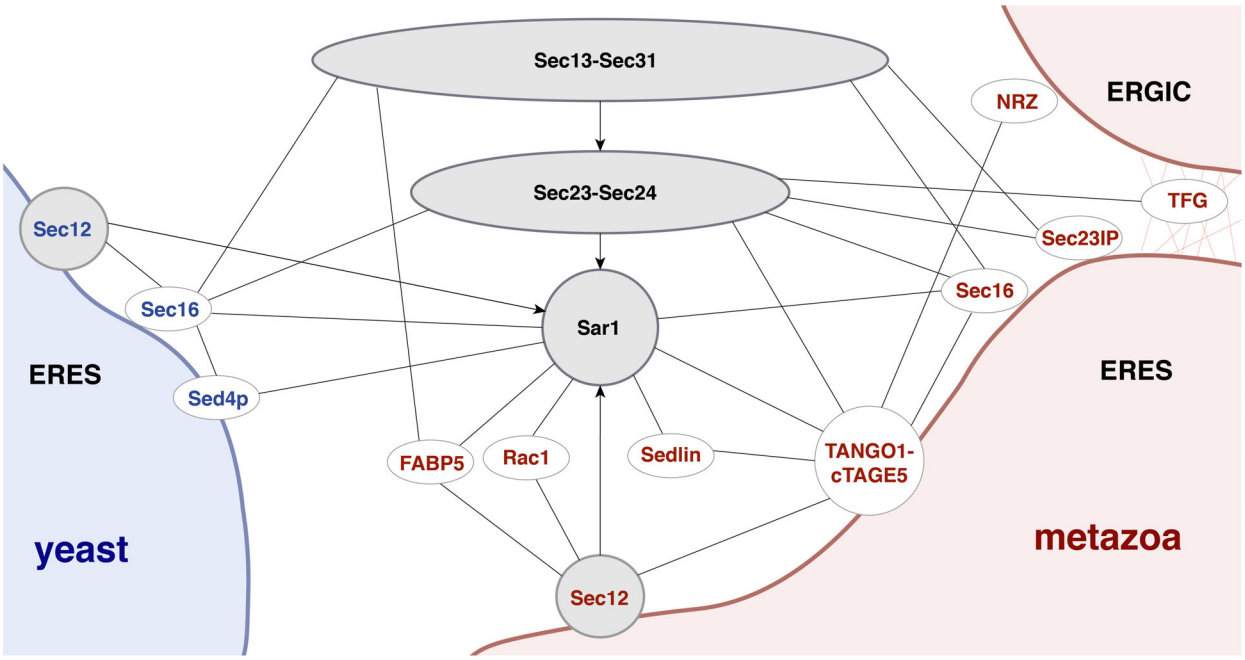


Fig. 4. Network of COPII interactions at ERES in yeast and metazoa. Central COPII machinery in grey circles with Sec12 activating Sar1 through its GEF activity, and Sec23-Sec24 and Sec13-Sec31 stimulating Sar1-GTP hydrolysis. Different sets of interactions have been shown for yeast (blue) and metazoa (red). Sec16 interaction with Sec12 has only been proven for *P. pastoris*. Metazoan interactions have been mostly described in humans and might deviate for other organisms, for example *Drosophila*, which has a pre-cis-Golgi rather than an ERGIC compartment and has no cTAGE5 as part of the TANGO1 complex.

vertebrates have two paralogues (Sar1A, Sar1B [17,50]) and plants have up to four (for a review, see [97]). This specialisation is also found in the other COPII components, with mammals having two Sec23, four Sec24 and two Sec31 paralogues. Based on transcriptome and proteome data in the Ensembl database [98], in humans, there is little tissue specificity for Sar1 expression, though Sar1A tends to be more broadly and highly expressed compared with Sar1B. Sar1A and Sar1B are 90% identical in sequence, with 8 out of 20 divergent residues located near the GTP-binding site and the Sec23-Sec31 interaction site [50]. As a result, Sar1B is found to have lower nucleotide exchange rates compared with Sar1A [50]. Furthermore, Sar1A and Sar1B show subtle differences in binding affinities for Sec23 and the Sec31 activating fragment [50].

Clues for the specialised functions of Sar1 paralogues come from disease data. Data in the literature point to Sar1B being specifically involved in secretion of large cargoes. Mutations in Sar1B result in the inability to secrete large lipoprotein particles and are linked to Andersons's and chylomicron retention disease (CMRD) [24]. In addition, two mutations in the inner coat subunit Sec23A near the Sec31-binding site (F382L, M702V) cause defects in the secretion of the large procollagen molecule, resulting in cranio-lenticulo-sutural dysplasia (CLSD). These Sec23A mutants show a defective behaviour in *in vitro* assays when used in conjunction with Sar1B, rather than Sar1A [44,45]. For both mutants, the ability to accelerate Sar1B GTPase activity is affected whilst remaining unchanged for Sar1A. In line with this, impairing Sar1B expression results in the retention of lipids and lipoproteins in fish and human cell lines [50,99].

The two Sar1 paralogues have also been implicated in COPII secretion-independent processes, such as fatty acid-binding protein 1 (FABP1)-mediated chylomicron secretion [100] and autophagy [101–103], although these roles of Sar1 are poorly understood and require further research.

Sar1 regulation mechanisms differ across species

In addition to the COPII coat components, other factors known to modulate Sar1-GTP activity and ERES organisation also have evolved specialised roles (Fig. 4). In *Saccharomyces cerevisiae*, Sed4p is an ER-resident homologue of Sec12 that has been implicated in COPII modulation [104], additionally interacting with Sec16 [57]. Sed4p does not act as GEF for Sar1 [104,105], instead, based on genetic evidence, it is

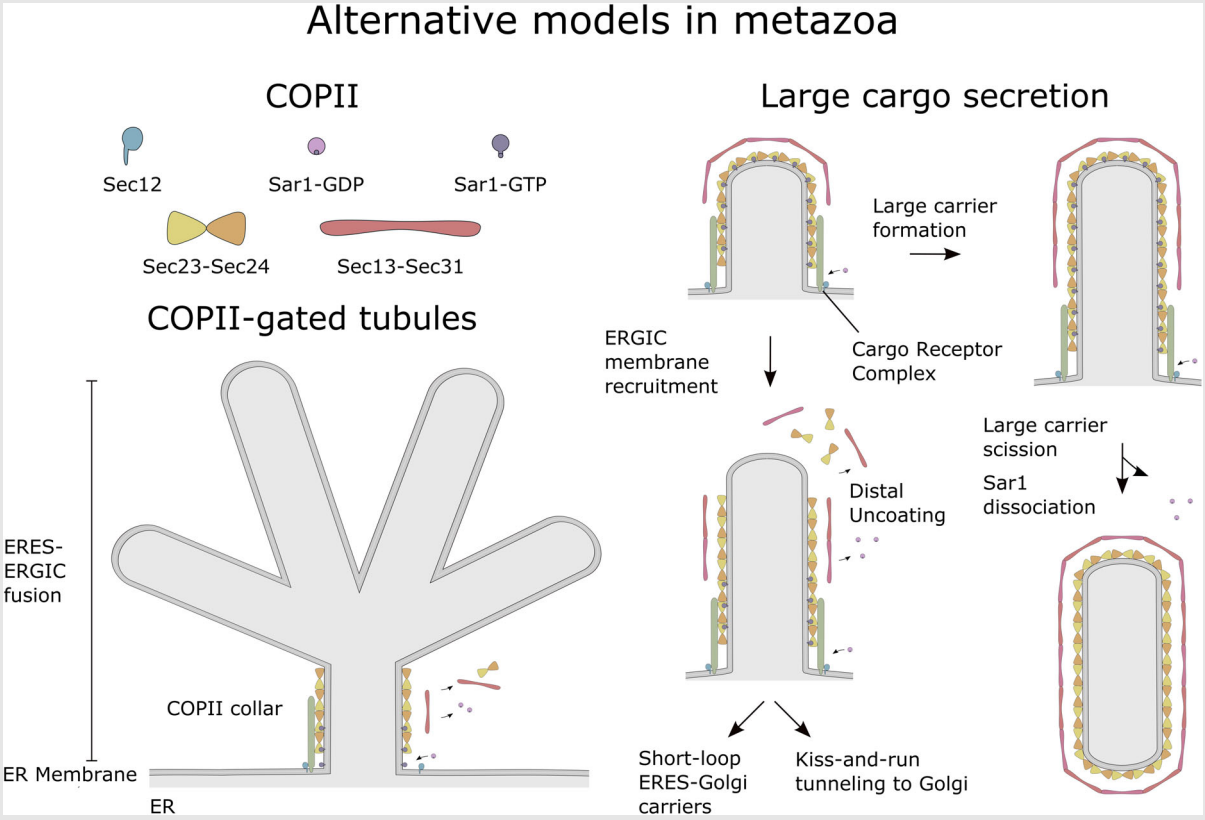
thought to increase the available Sar1 pool in a process also involving Sec16 [57,105]. Sed4p can bind cargo-free Sar1 and accelerate its GAP activity [104], possibly inducing dissociation of coat subunits not bound to cargo and thereby ensuring cargo concentration.

In human cell lines, recent research established the small GTPase Rac1 as a Sar1 interactor involved in the response to mechanical stress: through the formation of a Rac1-Sar1-Sec12 tripartite complex, ERES numbers transiently increase [106]. Finally, the mammalian protein p125, later dubbed Sec23IP (for Sec23 interacting protein) has also been shown to be involved in ERES organisation and cargo transport [107,108]. Sec23IP shows homology to phospholipase type A1 enzymes [109] and is capable of binding both Sec23 and Sec31 [108]. Sec23IP binds PI4P and recruits COPII proteins at the ERES downstream of Sec16 and in a Sec16-segregated process [110]. It is yet unclear how Sec23IP binding to COPII affects GTPase activity of Sar1. It is possible that by recruiting outer and inner coats, it could accelerate GTPase activity of Sar1 distally from the neck and favour uncoating.

Regulation in metazoa: the large cargo conundrum and the role of Sar1

In addition to the increased diversity of cargo throughout evolution, multicellular organisms bring additional challenges to the secretory machineries to sustain the build-up of the extracellular matrix and bulk lipid transport. As exemplified by the specialisation of A and B Sar1 paralogues, mechanisms have been developed to cope with these requirements, with additional factors modulating the COPII coat identified in metazoa (Fig. 4). The best-studied modulator of COPII transport is TANGO1, first discovered in Drosophila [111,112]. TANGO1 is a large transmembrane protein involved in the secretion of bulky cargo and the organisation of ERES [112–115]. Mammals have additional TANGO1-like regulators linked to large cargo transport such as cTAGE5 [116], TALI [117] and a short isoform of TANGO1, named TANGO1S [118]. Notably, the human genome codes for 7 more cTAGE variants, which might further expand functionalities [119]. The role of the cTAGE5-TANGO1 complex in the secretion of procollagen VII has been extensively characterised [112,116–118,120,121]. Both cTAGE5 and TANGO1 localise to the ERES [112,116] where TANGO1 interacts with Sec16 to act as a scaffold [114]. Furthermore, cTAGE5 binds and recruits Sec12 to efficiently activate Sar1 [122,123]. On the luminal side of the ER, TANGO1 binds procollagens via interaction of its SH3-like domain with procollagen chaperone HSP47 [124]. Different from most other cargo receptors, TANGO1 stays at ERES and is not transported towards the Golgi [112]. Interestingly, both TANGO1 and cTAGE5 bind Sec23 through triple-proline motifs in their cytosolic proline-rich domains (PRD) [112,116,125]. The binding site for the PRD [125] was later shown to correspond to the binding of the PRD of Sec31 to the inner coat [10], where it bridges between neighbouring coat subunits to stabilise the coat lattice. Therefore, TANGO1/cTAGE5 binding could compete with Sec31, leading to stabilisation of the inner coat by blocking Sec31 acceleration of GTP hydrolysis on Sar1 whilst promoting lattice assembly [9,10,125]. Despite a well-established dependence on COPII [119,126], the mechanisms of procollagen ER export remain debated. Studies using super-resolution light- and electron microscopy (EM) have suggested the existence of larger COPII-coated carriers capable of procollagen transport in human cell lines [127–129] and established their dependence on Sec31-ubiquitylation [128], TANGO1 and Sec12 [130]. In addition, carriers for procollagen IV independent of TANGO1 and ubiquitylation have also been observed [131]. Nevertheless, rapidly mounting evidence using multiscale approaches spanning state-of-the-art correlative light electron microscopy and focussed ion beam scanning electron microscopy (FIB-SEM) suggest that large cargo secretion of procollagens and lipoprotein particles happens in the absence of large COPII-coated carriers [67,132]. Instead, COPII is found confined to the base of buds in ERES, resembling a 'collar', and has been proposed to act as a general cargo sorting platform [67,133], with transport occurring either through short-loop carriers to the ERGIC [134], short-range ERES-ERGIC tunnelling [132,135] or via COPII-negative tubules [67,136] (Box 2). Interestingly, it has been suggested that the previously observed carriers [128–130] are instead degradation compartments, as ascorbate-induction of procollagen transport triggers autophagy [143]. The TANGO1-cTAGE5 complex seems the ideal candidate to scaffold a COPII collar. TANGO1-cTAGE5 complexes form ring-like structures [121,142,144,145] and can recruit ERGIC membranes through the binding of the NRZ-tethering complex [117,121]. Moreover, computational models support TANGO1 to act as a linactant, capping COPII lattices at the base of ER buds, allowing for stable neck regions and thus prolonged cargo loading, this as the result of a transient reduction in membrane tension [146]. Finally, by virtue of its peculiar transmembrane topology, the TANGO1-cTAGE5 complex is shown to act as a fence and limit lipid diffusion,

Box 2. Alternative COPII trafficking models in metazoa.



Sar1 gets activated by Sec12 [1] at the membrane and sequentially recruits the COPII coat components Sec23-Sec24 and Sec13-31 [2–4] (Fig. 1). According to the canonical model for anterograde trafficking, assembled COPII vesicles sized 60–100 nm detach from the membrane, sequentially uncoat and travel to the cis-Golgi compartment [2,68] (Fig. 1). The ERES and cis-Golgi are in close proximity in yeast and plants [20,137], though contact sites are only transient in *Saccharomyces cerevisiae* [138]. COPII-coated vesicles have indeed been clearly visualised in these organisms, providing direct evidence for the mainstream model [139–141]. It is instead unclear whether vesicular transport also occurs in metazoa and/or if it is the sole mode of transport. Recent developments have suggested COPII to act merely as a sorting collar regulating cargo entry in a fused ERES-ERGIC compartment [67,133]. Next, a tubular network of Rab1-dependent and COPI-positive membranes would transport cargo across the cell to the cis-Golgi ribbon [67,136]. Here, COPI-dependent scission might further help in sorting and delivering cargo [133]. For large cargo such as procollagen and lipoprotein particles, other secretion models have been proposed. Here, stabilisation of the inner COPII coat (Sar1-Sec23-Sec24) by large receptor complexes (depicted in green), such as those made by the cTAGE5-TANGO1 complex in the transport of procollagen VII, leads to the outgrowth of larger carriers [121,125]. These carriers are either released as full-blown COPII carriers [127–129] or recruit ERGIC membranes to form a short-range transport bridge [121,134]. The latter transport could be completed by short-loop delivery to the Golgi, where a carrier matures, and a COPII-negative or COPII-depleted carrier pinches off and travels a short way to the cis-Golgi [134]. Alternatively, fusion with the ERGIC-derived membranes could deliver cargo in a kiss-and-run mechanism directly yielding cargo to the Golgi in a short-range tunnelling mechanism [121,135,142]. These tunnels have also been imaged between the ERES and pre-cis-Golgi (functionally equivalent to ERGIC) in Drosophila, though their nature is unclear [132].

thus preventing membrane mixing from these two different compartments of the cell [147], possibly helping to concentrate Sar1 molecules.

In addition to the TANGO1-like family of proteins, other Sar1 modulators have been described that affect large cargo secretion. Sedlin, part of the TRAPP complex, is recruited to COPII budding sites by interaction with TANGO1 and preferentially binds to Sar1-GTP molecules [148]. Sedlin depletion inhibits procollagen export, and it might act by sequestering Sar1-GTP from the budding carrier, precluding constriction and eventual scission [148]. Fatty acid-binding protein 5 (FABP5), involved in the binding and transport of fatty acids, has also been implicated in lipoprotein and collagen secretion [149]. FABP5 binds Sec12 increasing Sar1-GTP-loading, and bridges between Sar1 and Sec31, possibly precluding inner coat binding, thus stabilising Sar1-GTP at the COPII collar to form large vesicles [149].

Finally, post-translational modifications of Sar1 interacting proteins have also been found to alter secretion patterns, such as ubiquitylation of Sec31 [128,150], phosphorylation of Sec23-Sec24 [68,151], deglycosylation of human Sec24 [151] and dephosphorylation of Sec31 [152], showcasing the importance of PTMs in tuning COPII transport. Given the difficulty in analysing PTMs in this complex process it is likely we have only scratched the surface of the mechanisms at play.

Conclusions and perspectives

Sar1 has a well-established role in the COPII-mediated export of newly synthesised proteins from the ER. However, aspects of its mechanism during membrane budding and scission are still poorly understood. Sar1 binds a plethora of factors that contribute to its function and regulation in unclear ways. Even less is known about the potential roles of Sar1 in other processes such as autophagy and growth regulation and their relationship with the early secretory pathway.

Here we have reviewed the literature on Sar1, focussing on three main aspects:

1 The role of Sar1 and GTP hydrolysis in coat assembly and disassembly. Whilst the GAP activity of Sec23-Sec24 and Sec13-31 have been well characterised, the effects of cargo, additional proteins and their modifications on Sar1-GTPase activity and the resulting effect on COPII-mediated secretion are still poorly understood, as testified by the plethora of models that have been proposed (Box 2).
2 The mechanisms of membrane deformation have been extensively studied using biophysical, biochemical and cellular approaches, and several models have been proposed. Whilst we have reviewed these models individually as they were introduced in the literature, all these mechanisms could act together or at different stages of the membrane remodelling process. A recurring theme in these models is that Sec12 is key in the activation of Sar1 at ERES to orchestrate secretion in a spatio-temporal manner. As such, localised activation of Sar1 plays an important role in membrane budding and scission events, though the requirement of GTP turnover in these processes is still unclear.
3 The complexity of Sar1 regulation increases significantly throughout evolution, as more proteins are found to interact with the known components and influence their activities. We reviewed a number of factors that have evolved specialised roles and are crucial in secretion in multicellular organisms. It is clear that more work will be needed to understand the full complexity of Sar1 role in ER export, both for standard and bulky cargo, especially in multicellular organisms. Exploiting cutting-edge technological advancements in fluorescence and electron microscopy imaging, we will likely be making significant steps forward in understanding how metazoan transport is regulated in the coming years.

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