Review

Golgi reassembly after mitosis: The AAA family meets the ubiquitin family

Hemmo H. Meyer*

Swiss Federal School of Technology (ETH), Institute of Biochemistry, ETH Honggerberg HPM, 8093 Zurich, Switzerland

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Abstract

The Golgi apparatus in animal cells breaks down at the onset of mitosis and is later rebuilt in the two daughter cells. Two AAA ATPases, NSF and p97/VCP, have been implicated in regulating membrane fusion steps that lead to regrowth of Golgi cisternae from mitotic fragments. NSF dissociates complexes of SNARE proteins, thereby reactivating them to mediate membrane fusion. However, NSF has a second function in regulating SNARE pairing together with the ubiquitin-like protein GATE-16. p97/VCP, on the other hand, is involved in a cycle of ubiquitination and deubiquitination of an unknown target that governs Golgi membrane dynamics. Here, these findings are reviewed and discussed in the context of the increasingly evident role of ubiquitin in membrane traffic processes.

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1. Introduction

During cell division, the Golgi apparatus in animal cells undergoes dramatic morphological changes. At the onset of mitosis during prometaphase, the perinuclear ribbon of Golgi stacks disassembles into fragments, Golgi components are dispersed throughout the duplicating cell and are then reassembled at the end of telophase to form a functional organelle in the two daughter cells [1]. The exact fate of Golgi components during anaphase and the mechanisms of biogenesis of the new Golgi apparatus is still a matter of controversy [2–4]. Experiments that followed the localization of Golgi enzymes pointed to a redistribution of this type of components into the ER, whereas other results exclude the possibility of complete mixing [5,6]. Further evidence suggested that Golgi remnants remain as discrete entities throughout mitosis. These mitotic Golgi clusters consist of an array of vesicles and tubules that contain Golgi matrix proteins and that serve as templates for the reformacion of the new Golgi apparatus [7–10].

Golgi reformation first involves the transport and concentration of membrane carriers that contain Golgi components to the site of Golgi formation. Reassembly of Golgi stacks then requires membrane tethering, stacking and fusion mechanisms that are also employed during its function for the transport of secretory material, as well as additional regulatory mechanism that are specific to mitosis. For example, a tethering complex composed of GM130 on cisternae, giantin on vesicles and soluble p115 ensures efficient vesicular fusion in interphase [11]. Its inactivation by phosphorylation of GM130 in mitosis contributes to Golgi fragmentation. Dephosphorylation of GM130 in telophase then initiates Golgi reformation by tying the mitotic fragments together [12,13]. Likewise, mitotic phosphorylation also regulates the function of the cisternal stacking factor GRASP65 [14,15,135]. SNARE (soluble NSF attachment protein receptor) proteins that mediate most, if not all, membrane fusion steps in the cell are also required for postmitotic Golgi cisternal regrowth. A lot of effort has therefore been focused on understanding the regulation of SNARE activity in the process. Two related AAA ATPases, NSF (N-ethylmaleimide sensitive factor) and p97/VCP (valosin-containing protein) have been implicated. NSF...
dissociates SNAREs from complexes that form during membrane fusion [16,17] and p97 was believed to act similarly. However, recent reports that revealed links to the ubiquitin system have questioned this model. The aim of this article is to review these data and reopen the discussion concerning the underlying mechanisms of NSF- and p97-mediated membrane fusion during Golgi reassembly.

2. NSF and p97/VCP regulate different membrane fusion events during Golgi reassembly

The pivotal role of NSF in membrane fusion was first identified in a cell-free system that reconstitutes intra-Golgi transport [18–20]. Similarly, a cell-free system that reproduces the mitotic Golgi disassembly–reassembly cycle in vitro has been instrumental to deconstruct the molecular mechanisms underlying mitotic regulation of Golgi architecture [21,22]. In this system, highly purified rat liver Golgi stacks are incubated in mitotic cytosol from synchronized HeLa cells to generate mitotic Golgi fragments that consist of vesicles and short tubules (Fig. 1). After re-isolation and upon incubation with interphase cytosol, these fragments reassemble into morphologically intact Golgi stacks, thereby demonstrating the inherent capacity of self-organization of this organelle [23].

The system has been used to identify the cytosolic components for the reformation process. Two reassembly pathways were identified. Not unexpectedly, the first requires NSF together with its cofactor alpha-SNAP (soluble NSF attachment protein) and the tethering factor p115. These components generate Golgi stacks but with unusually short cisternae. However, another cytosolic AAA ATPase, p97 that had no known function at that time also generated Golgi cisternae together with its cofactor p47 [24,25]. These cisternae are much longer than those generated by the NSF pathway, but they can also be stacked when p115 is added [26]. Both pathways assemble about the same amount of membranes into cisternae, but the activities are not additive [25]. Only the two pathways together can substitute for cytosol activity and generate morphologically intact Golgi stacks with long cisternae.

A differential role of the two pathways was also observed in a Golgi reformation system that monitors the reassembly of Golgi stacks in permeabilized, semi-intact cells after Golgi fragmentation into small vesicles with the drug ilimaquinone [27]. Whereas NSF mediates membrane fusion that leads to the generation of large vesicles, p97 is required in this system to generate long cisternae that can then associate to Golgi stacks [28].

A series of reports have identified activities of p97 and its orthologs in other organelle fusion events and have thereby supported a general role for p97 in organelle reformation and maintenance. In yeast, the p97 orthologue Cdc48p mediates fusion of ER membranes [29]. In vertebrates, p97 is also needed for the assembly of transitional elements of the ER [30] and ER network formation [31]. Finally, reformation of the nuclear envelope after mitosis requires p97 in Xenopus egg extracts [31]. Intriguingly, in this process, p97 mediates two separate reactions with different cofactors that may represent distinct membrane fusion events [32]. Together with its heterodimeric cofactor Ufd1–Npl4 [33], p97 is needed for the fusion of chromatin-bound vesicles that leads to the initial sealing of the nuclear envelope. The subsequent expansion of the nuclear envelope depends on the feeding of additional membrane material and requires p97 together with p47, indicating that there are similarities to Golgi fusion.

These findings and the absence of any evidence for a role of p97 in vesicular transport of the secretory pathway [34] suggested that p97 might preferentially regulate homotypic membrane fusion between equivalent organelle fragments. Conversely, NSF would mediate heterotypic fusion of vesicles with target organelle membranes, which is also needed for postmitotic Golgi reassembly [22]. This classification is not strictly valid, though, since NSF is required for the homotypic fusion of immature secretory granules [35] and its orthologue Sec18p in yeast is needed for fusion between vacuoles [36]. The differential requirements for the two AAA ATPases must, therefore, have a different molecular basis.

3. NSF and p97/VCP: cousins with different characters

Both NSF and p97 belong to the AAA (ATPases associated with different cellular activities) protein family whose members exist in all organisms (for review see [37,38]). AAA proteins share a common domain of about 230 amino acids that contains the Walker A and B motifs, critical for nucleotide hydrolysis and binding, respectively, and a second region of homology. In general, AAA ATPases use the chemo-mechanical energy generated by ATP hydro-
lysis for structural remodeling of substrate proteins. Different AAA proteins use this activity for specific tasks including protein complex assembly or disassembly, disaggregation or the complete unfolding of substrate proteins. For simplicity, it will be referred to as chaperone activity in the following text.

NSF and p97 both have two AAA domains (D1 and D2) that are stacked and arranged as a hexamer (Fig. 2) [39–41]. They also possess a related amino-terminal (N)-domain that consists of two subdomains (Na and Nb) separated by a cleft. The N-domains are positioned at the periphery of the hexamers in a plane with D1. They are required for binding of substrates and cofactors. Work on p97 has revealed intramolecular movements upon nucleotide hydrolysis and exchange [42–44] that include opening and widening of the central pore, rotational forces and movements of the N-domain. These movements are likely transmitted, directly or indirectly, onto substrate proteins to apply mechanical force.

Despite these similarities, however, recent work has revealed considerable differences between the two proteins that point to distinct mechanisms of action (reviewed in [45]), only a few of which can be highlighted here. Probably, the most significant among these is the differential use of the two AAA domains. In NSF, D1 has the main ATPase activity, whereas D2 is only required for hexamerisation [46,47]. The opposite is the case for p97, where D2 has a dominant activity [48,49]. ATP hydrolysis in D1 may depend on the activity in D2 through a proposed allosteric communication between the domains [41,49]. However, the function of ATPase activity in D1 is unclear, since it is not essential [50]. Moreover, structural work suggests that, contrary to p97, the D2 in NSF is arranged ‘tail to tail’ with D1 [51], further supporting the notion that force generation and transmission is very different in the two ATPases. Finally, also substrate interaction appears to be dissimilar, since proposed substrate interaction regions in the Na subdomain or the cleft of the N-domain have different surface properties [45]. Consistent with this is the observation that p97 appears to have a broader chaperone activity, since it is able to bind unfolded proteins and prevent their aggregation [49,52]. Together, these recent advances strongly suggest that NSF and p97 use distinct mechanisms to catalyze protein remodeling processes.

4. The classical role of NSF

The central players in intracellular membrane fusion events are SNARE (SNAP receptor) proteins ([16,53–55] and references therein). About 30 SNAREs exist in mammals that generally reside and fulfill their functions on specific membranes. SNAREs have been classified in vesicular transport processes into v- and t-SNARE according to their localization on vesicle (v) or target (t) membranes. During membrane fusion, helical sections of v- and t-SNAREs on apposing membranes bind and form a trans-SNARE complex or SNAREpin. SNAREpin formation is best understood at the synapse [56]. Here, the t-SNARE on the pre-synaptic membrane consists of syntaxin-1 [57] that contributes one heavy chain, and SNAP-25 [58] that contributes two helical light chains. The v-SNARE Vamp/synaptobrevin [59,60] on the vesicle has a single helical chain. Upon binding, v- and t-SNAREs coil up to form an unusually stable parallel four-helix bundle. This binding brings the apposing membranes into close proximity probably overcoming the repulsive forces between them, and either trigger lipid bilayer fusion.

![Fig. 2. Structure of the NSF and p97 AAA ATPases.](image-url)
[61] or signal to downstream events [62]. Pairing of individual SNAREs contributes to the specificity of fusion events. However, pairing is further regulated by additional mechanisms that involve a variety SNARE binding proteins, including the heterodimer LMA-1 or the SM-proteins, Sec1p, munc18, sly1p and others (reviewed in [54,63]). Often, the exact function of these regulators is unclear, but different factors can either inhibit or stimulate SNARE binding, or contribute to its specificity.

As a result of fusion, all SNAREs in the SNAREpin are localized in a cis-SNARE complex on the fused membrane. This very stable, often SDS-resistant protein complex is dissociated by the action of NSF in order to recycle the individual SNAREs for further rounds of fusion [17,64]. NSF binds via its cofactor alpha-SNAP to the complex and unravels SNAREs using the mechano-chemical energy generated by ATP hydrolysis. Although many details remain to be clarified, cis-SNARE complex dissociation is probably the best example for segregation of a highly stable protein complex catalyzed by a AAA ATPase.

5. SNARE complex dissociation is not required during Golgi reassembly

The Golgi apparatus contains several SNAREs that have specific roles in individual vesicular transport steps. During Golgi reassembly, at least two of them, syntaxin-5 and gos-28/GS28, are involved in NSF-mediated fusion [65]. Syntaxin-5 is predominantly found on Golgi cisternae, whereas gos-28 is enriched on vesicles [11]. This suggests that pairing of the two proteins on apposing membranes promotes fusion. Reassembly mediated by p97, on the other hand, involves syntaxin-5, but is independent of gos-28 [65]. Binding studies with recombinant, GST-tagged syntaxin-5 hand, involves syntaxin-5, but is independent of gos-28 [65].

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6. p97 is a ubiquitin-dependent chaperone

Novel insights into how p97 may act mechanistically first came from data on other cellular processes. Unlike NSF, p97 is very abundant (an estimated 1% of cytosolic protein) and is required in a large variety and still increasing number of different cellular pathways. Apart from organelle membrane fusion, these functions range from bulk degradation of ER proteins to specific regulation of cell cycle progression, gene expression and apoptosis [70]. It is reasonable to assume that in each case, p97 functions as a chaperone or remodeling factor that unfolds or dissociates proteins and their complexes. This activity, however, appears to be modulated by distinct sets of mutually exclusive adapters and additional cofactors that divert p97 function to different processes [33]. How these factors modulate p97 function is not fully understood, but an example is the fact that p47 regulates the ATPase activity of p97 [71].

Besides p47, a large number of cofactors and binding proteins have been isolated in different species [70]. The best-characterized adapter is the conserved Ufd1–Npl4 heterodimer [33]. Both p47 and Ufd1–Npl4 bind p97 in a mutually exclusive manner, which is based on a common bipartite binding mechanism [72–75].

The p97–Ufd1–Npl4 complex itself mediates different reactions, which may, however, be mechanistically related. One of the better-understood functions is its role in ER-associated degradation (Fig. 3) [76,77]. Short-lived or misfolded proteins that reside in the membrane or lumen of the ER have to be transported into the cytosol, where they
are degraded by the ubiquitin–proteasome system. In general, ubiquitin-dependent degradation is initiated by the covalent conjugation of ubiquitin to lysines of substrate proteins [78]. This is mediated by a catalytic cascade that includes an activating enzyme E1, E2 conjugating enzymes and distinct, substrate-specific E3 ubiquitin ligases. Sequential ubiquitin conjugation to lysine-48 of the previous ubiquitin unit generates chains. Chains linked via ubiquitin residue lysine-48 constitute a degradation signal (B) Role of p97 and its adapter Ufd1–Npl4 (UN) during ER-associated degradation. ER proteins destined for degradation are ubiquitinated at the cytosolic face of the membrane. p97 and Ufd1–Npl4 bind to the substrate via unfolded segments within the peptide and through interaction with the ubiquitin chain. ATP hydrolysis by p97 induces mobilization of the substrate into the cytosol for subsequent degradation by the proteasome.

7. p97–p47-regulated Golgi dynamics during mitosis is governed by a cycle of ubiquitination and deubiquitination

Originally, it was postulated that, in contrast to the Ufd1–Npl4 adapter, p47 was an adapter that conferred a ubiquitin-independent role to p97 [33]. However, recent evidence showed that p97–p47 activity also requires ubiquitin, albeit in a proteasome-independent manner. The involvement of ubiquitin first became evident with the discovery of a ubiquitin-associated (UBA) domain at the amino terminus of p47 [86,88]. UBA domains are short helical ubiquitin-interaction modules that are found in many other proteins [89–91]. Like the Ufd1–Npl4 adapter, p47 can bind ubiquitin and recruit p97 to a ubiquitinated protein that likely also is the substrate of its chaperone activity. In contrast to Ufd1–Npl4, however, p97–p47 can only be recruited as a whole and not sequentially, since p47 only binds ubiquitin when in complex with p97 [86]. Importantly, a functional UBA domain is essential for p97–p47-mediated Golgi reassembly, since either deletion of the domain or a single point mutation that abolishes ubiquitin-binding, significantly decreases reassembly activity [86]. The obvious conclusion that p97–p47 recognizes and processes a ubiquitin-conjugate on the membrane was further supported using a dominant-negative ubiquitin mutant with an isoleucine-44 to alanine exchange (I44A) that strongly inhibits Golgi reformation [92]. Like most, if not all ubiquitin interaction domains, the UBA domain binds ubiquitin at a hydrophobic patch around isoleucine-44 [93]. Mutation of this residue still allows conjugation, but the resulting conjugate on the Golgi membrane cannot be recognized by downstream factors and therefore processing by p97–p47 is blocked.

The nature of the ubiquitinated target on the Golgi membrane is still unclear, but the experiments with ubiquitin(I44A) have revealed another aspect. The mutant only blocks reformation when added before Golgi disassembly is completed, and it has no effect when added later during reformation. This indicates that a critical ubiquitina-
tion step occurs already during mitotic fragmentation. The fact that p97–p47 is inactivated at the same time could explain why the ubiquitin-conjugate remains stable throughout mitosis. Phosphorylation of p47 at serine-140 by Cdk1 removes the complex from the membrane early in mitosis [94]. Dephosphorylation in telophase allows rebinding and subsequent processing of the ubiquitinated target (Fig. 4).

Ubiquitin not only triggers degradation of substrates by the proteasome, but can also regulate proteasome-independent processes [95]. In these cases, substrates are either modified with a single ubiquitin (monoubiquitin) or with short chains that are linked via lysine-63 instead of lysine-48 of the previous ubiquitin unit. Several lines of evidence showed that p97–p47-mediated Golgi reassembly involves monoubiquitination and is proteasome-independent. First, in vitro binding experiments revealed that the p97–p47 complex has a preference for binding to monoubiquitin rather than to polyubiquitin when compared to Ufd1–Npl4 [86]. This does not exclude that some p47-mediated processes can involve polyubiquitination. At least in yeast, p47 homologues appear to be linked, directly or indirectly, to degradative processes [96,97]. As for p97–p47-mediated Golgi reassembly, however, neither a ubiquitin(K48R) mutant that abolishes lysine-48 chain extension and therefore proteasome targeting, nor proteasome inhibitors have any effect on cisternal regrowth, showing that proteasomal degradation is not involved [92]. Furthermore, p97–p47 activity is dependent on another cofactor, VCIP135, which resides on the membrane and can form a transient complex with p97–p47 via a ubiquitin-fold domain [73]. VCIP135 belongs to the OTU (ovarian tumor) type of deubiquitinating enzymes that share a homologous catalytic domain with cysteine protease activity [92,98,99]. Deubiquitinating enzymes are isopeptidases that reverse the activity of ubiquitin ligases by cleaving the bond between ubiquitin and its target. They therefore act analogously to phosphatases in removing a signal from a protein. Indeed, VCIP135 deubiquitinating activity is required for Golgi reformation, indicating that removal of the ubiquitin signal generated during Golgi fragmentation is an essential step in the p97-mediated mechanism that triggers membrane fusion [92].

These new findings have outlined a cycle of ubiquitination during the Golgi disassembly and deubiquitination during reassembly that governs the p97-mediated pathway (Fig. 4). The exact underlying mechanism, the identity of the targets in this pathway and how they mediate cisternal regrowth, however, still need to be clarified. It is conceivable that ubiquitin serves as a recruitment signal for p97–p47, which then applies its chaperone activity to fusion regulators. In some analogy to the role of p97–Ufd1–Npl4, the p97–p47 complex could also extract a ubiquitinated inhibitor of fusion from the membrane. Alternatively, ubiquitin itself could serve, directly or indirectly, as a regulator that need to be recognized and then removed by p97 and its cofactors. The multitude of possibilities become apparent if one looks at the diverse mechanisms that are deployed by ubiquitin to regulate other membrane trafficking events.

8. Ubiquitous roles of ubiquitin in membrane trafficking

In the proteasomal degradation pathway, the function of the modification with lysine-48 linked ubiquitin chains is believed to be the targeting of ubiquitinated substrates to the proteasome. This is achieved by direct interaction of the ubiquitin chain with proteasomal components or with factors that transiently bind to the proteasome. In contrast, the molecular consequences and functions of monoubiquitination or lysine-63 chains in proteasome-independent processes appear to be more diverse, although downstream events also involve physical interaction of ubiquitin with specific ubiquitin binding domains. Many membrane trafficking processes are regulated by these types of ubiquitination and do not involve the proteasome. Since they have been reviewed in excellent recent publications (for example [100–102]), only a few examples will be mentioned to illustrate mechanistic concepts that are relevant for this discussion.

The best-studied ubiquitin-regulated trafficking steps are within the endocytic pathway, namely the internalization of certain plasma membrane receptors and transport of cargo from the sorting endosome to the multi-vesicular bodies for subsequent degradation in the lysosome. In both cases, transport cargo is modified with ubiquitin, which then constitutes a sorting signal (reviewed in [103,104]). This signal functions by directly interacting with short ubiquitin-
interacting domains in factors that mediate transport to the appropriate location. These factors include epsin and Eps15 at the plasma membrane and Hrs/Vps27 at the endosome. All three of them contain a ubiquitin-interacting motif (UIM) that binds the ubiquitinated cargo and integrates them with lipid and clathrin interactions, which mediates sorting and transport. Further transport from the endosome to the internal membranes of the multi-vesicular bodies requires the subsequent interaction with three protein complexes, in yeast called ESCRT I–III. Again, recruitment is mediated via direct interaction with the ubiquitin moiety. ESCRT-I is recruited to the cargo via the ubiquitin-binding UEV domain in one of its components, Tsg101/Vps23 [105,106] and, at least in yeast, ESCRT-II via a NZF domain in Vps36 [107]. It is noteworthy that this pathway also requires a AAA ATPase, Vps4, that in this case is believed to recycle the sorting factors from the membrane [106,108]. Furthermore, it also involves a deubiquitinating enzyme, Doa4p, which removes the ubiquitin signal from the cargo to recycle it before the cargo moves with the budding membrane into the lumen of the MVB for degradation [106,109–111]. In mammals, at least two deubiquitinating enzymes are involved in the process, UBPY [112,113] and AMSH [114].

These examples show that ubiquitination as a recruitment and sorting signal appear to be a common theme. However, further work on epsin and Eps15 also revealed a different concept. As well as containing a UIM, they are also themselves ubiquitinated [115,116]. This may allow the formation of a network of interactions between sorting factors and the cargo that could define and regulate an active region on the membrane. Intriguingly, a functional UIM is also a prerequisite for ubiquitination of both epsin and Eps15. The mechanistic reason for this is still unclear, but it is likely to control the formation of ubiquitin–UIM interactions that regulates endocytosis. Ubiquitin-mediated interactions between these factors appear to be dynamic. Data from Drosophila indicate that a deubiquitinating enzyme termed fat facets regulate endocytosis by deubiquitinating the epsin homolog liquid facets [117].

An intra-molecular bridge between the UIM of epsin or Eps15 and the ubiquitin moiety on the same molecule could also directly regulate their activity. A similar model has also been proposed in the case of Vps9. Vps9 is the ortholog of mammalian Rabex-5 and acts as a GEF for the rab5-like Vps21 [118,119]. Vps9 contains a ubiquitin-binding CUE (Cue1-homologous) domain, but Vps9 is also itself ubiquitinated [120,121]. Intriguingly, the CUE domain is not required for Vps9 function in endocytosis. The available data, however, have led to a model in which Vps9 is normally inhibited by an intramolecular bridge between the CUE domain of Vps9 and its ubiquitin moiety. This auto-inhibition would be alleviated only if receptor-conjugated ubiquitin binds and occupies the CUE domain. In that case, Vps9 GEF activity would be restored and could stimulate GDP to GTP exchange in Vps21, which ultimately triggers endosomal membrane fusion.

These examples illustrate the complexity of ubiquitin-dependent regulation in membrane trafficking. They show how ubiquitin ligases, deubiquitinating enzymes and AAA proteins can be important regulators in these pathways. The diversity of molecular mechanisms that are deployed is still likely to grow. As if this was not enough, ubiquitin-like modifiers have also been implicated in membrane dynamics. The most relevant for this discussion is GATE-16, which is involved in NSF-mediated membrane fusion in the Golgi apparatus.

9. NSF-mediated fusion involves the ubiquitin-like protein GATE-16

GATE-16 was originally isolated biochemically based on its activity to stimulate vesicular Golgi transport in a cell-free assay [122]. It physically interacts with both NSF and the Golgi v-SNARE gos-28 and experiments with isolated proteins suggested that NSF catalyzes the complex formation between GATE-16 to gos-28 (Fig. 5). When bound to GATE-16, gos-28 cannot bind its cognate t-SNARE component syntaxin-5, strongly suggesting that GATE-16 acts as a regulator of SNARE pairing [123]. Importantly, NSF-mediated regulation of GATE-16 to gos-28 binding (and therefore regulation of SNARE pairing) requires ATP, but is independent of ATP hydrolysis [68]. Since GATE-16 acts also for NSF-mediated reassembly of mitotic Golgi fragments [68], it is attractive to speculate that NSF and GATE-16 regulated SNARE pairing accounts for the ATP-hydrolysis independent function of NSF during Golgi-reformation after mitosis.

GATE-16 belongs to a subfamily of ubiquitin-like proteins that among others include MAP1-LC3 in mammals and Atg8p in yeast (formerly Apg8p or Aut7p [124]). The common structural feature that distinguishes these proteins from other ubiquitin-like proteins is an additional helical region at the N-terminus that folds back on the beta-sheet surface of the ubiquitin-fold [125]. Atg8p is essential for autophagy, a process that involves the

Fig. 5. NSF has two separate functions during mitotic Golgi membrane dynamics. NSF binds via alpha-SNAP to cis-SNARE complexes and dissociates them in a reaction that requires ATP hydrolysis. NSF then mediates complex formation between the v-SNARE gos-28 (green) and the ubiquitin-like protein GATE-16 (G16). GATE-16 regulates pairing of gos-28 with the t-SNARE syntaxin-5 (orange), that leads to membrane fusion. Whether membrane anchoring through lipidation of GATE-16 plays a role in this process is unknown.
wrapping of large parts of the cytoplasm with membrane cisternae (for review, see [126]). These cisternae form the autophagosomal that then fuses with the lysosome to allow digestion of the entire structure. Like ubiquitin, Atg8p is processed by a cysteine protease, Atg4p to expose a C-terminal glycine [127]. In contrast to ubiquitin, however, it is not transferred onto a protein but to phosphatidyl-
ethanolamine, thereby modifying membrane rather than proteins [128]. This process is mediated by the E1-like activating enzyme Atg7p and the E2-like conjugating enzyme Atg3p. The mechanistic role of Atg8p has remained elusive. In analogy to the role of GATE-16, it may be involved in the regulation of one of the required membrane fusion steps during autophagy. This is supported by the finding that it interacts with the SNAREs Bet1p and Nvy1p [129].

In mammals, MAP1-LC3 is involved in autophagy, whereas the other family members may have diverged functions [126]. All members undergo processing and appear to be lipidated, but in the case of GATE-16 also the non-modified form is membrane associated [130]. Therefore, it is not known yet whether lipidation is required for GATE-16 function in Golgi membrane fusion. An attractive model would include lipidation as part of regulation of SNARE pairing and perhaps even SNARE-mediated membrane fusion. Intriguingly, human App4p that processes the C-terminus [131,132] can also de-lipidate Atg8 homologs [133], analogously to the function of a deubiquitinating enzyme in the ubiquitin system. This raises the possibility that a cycle of lipidation and de-lipidation could be an integral part of NSF and GATE-16 regulated membrane fusion. This, in turn, would have intriguing similarities to the ubiquitin-cycle that is required in the pathway mediated by p97–p47 and the deubiquitinating enzyme VCIP135.

10. Conclusions and outlook

Reformation of the Golgi apparatus after mitosis requires membrane fusion mechanisms that are common to vesicular transport steps in the secretory pathway during interphase. However, it also requires additional mechanisms that coordinate the morphologically correct assembly and that orchestrate the process with other events during exit from mitosis including chromosome segregation, reorganization of microtubules, nuclear reformation and cytokinesis.

Membrane fusion depends on SNARE proteins and first requires dissociation and reactivation of SNAREs from preexisting cis complexes. Biochemical dissection of mitotic Golgi dynamics has revealed that, like in all other confirmed cases, cis-SNARE complex dissociation is mediated by NSF and its cofactors and requires ATP hydrolysis. These analyses also uncovered that NSF has a second function that is independent of ATP hydrolysis. An attractive model is that this activity accounts for its role in mediating binding of the ubiquitin-like modifier GATE-16 to the v-SNARE gos-28. GATE-16 regulates v- and t-SNARE pairing both during Golgi reformation and during Golgi transport. This second function will certainly stimulate further investigation. It will also be important to identify the other SNAREs involved Golgi reassembly to get a complete picture.

Meanwhile, the work on the role of NSF has already clarified that SNARE dissociation cannot account for the role of the other identified regulatory pathway mediated by p97 and its cofactors. In contrast to NSF, current available data do not support a role for p97 in the secretory pathway. Therefore, one possibility is that it controls the assembly of a subset of mitotic fragments that constitute the organelle ‘core’, which does not undergo fission and fusion during interphase transport. Since this first model was proposed [25], however, we have learned a lot on the role of p97 in the ubiquitin system and its regulation of other post-mitotic events including spindle disassembly and nuclear reformation. Based on these new findings, it is also possible that the p97 pathway might simply provide an additional layer of regulation that controls Golgi membrane fusion to ensure the timely reassembly of the Golgi apparatus after mitosis.

It will require further investigation to clarify what model holds true. Meanwhile, recent progress has outlined the major events in the pathway, which will help further approaches (Fig. 4). The pathway involves a ubiquitination step that is mediated by an unknown ubiquitin ligase during Golgi fragmentation. At about the same time, phosphorylation of p47 by Cdk1 removes p97–p47 from the membranes and thereby inactivates it. Later, during exit from mitosis, loss in Cdk1 activity allows dephosphorylation of p47 and recruitment of p97–p47 to the site of ubiquitination via the UBA domain in p47. Consequently, p97 can then exert its chaperone activity on a putative substrate. The cycle is concluded by VCIP135 that removes ubiquitin and ultimately triggers membrane fusion.

Based on the general ubiquitin-dependent chaperone activity of p97 known from unrelated cellular pathways and the variety of functions of ubiquitin in other membrane trafficking steps, several scenarios of how this may regulate Golgi reassembly are conceivable (Fig. 6). One possibility is that p97 is recruited via ubiquitin to a SNARE or a mitotic SNARE regulator and then triggers conformational changes that are required for SNARE pairing. The fact that p47 can bind syntaxin-5, whose function is required for p97-mediated fusion supports this notion. However, also factors that act upstream of the SNARE-mediated step, particularly tethering factors, could be potential targets of the pathway. Alternatively, p97 could extract a mitotic SNARE regulator or simply remove ubiquitin that itself could act as an inhibitor of SNARE pairing. It becomes clear that any further understanding of the underlying mechanisms and of the cell biological relevance of the p97 pathway will depend on the identification of its target and of the E3 ligase that
It is therefore not unlikely that the NSF and p97 revealed that it can cross-talk with the ubiquitin system ubiquitin-like modifier SUMO in unrelated processes linking the NSF and p97 pathways. Work on the p97 function for Golgi reformation in vivo, further experiments will hopefully clarify the relevance of inhibition of p97 function in these experiments. Therefore, infected. This points to an efficient, yet incomplete formation and spindle disassembly were seemingly unaffected. Wherever NSF(E329Q) caused disassembly of the Golgi ribbon, no effect was observed with p97(E578Q). However, some aspects need to be considered when interpreting these results. For example, ultrastructural analysis of the extent of vesiculation of the Golgi after mitosis was undertaken in p97(ES578Q) expressing cells. Furthermore, although p97–Ufd1–Npl4 clearly requires ATP hydrolysis for its function, it has not been established whether this is true for p97–p47. The p97(ES578Q) mutant may therefore be functional during Golgi reassembly. And finally, although an effect on the predominant role of p97–Ufd1–Npl4 in ER-associated degradation was observed, other roles during exit from mitosis including nuclear formation and spindle disassembly were seemingly unaffected. This points to an efficient, yet incomplete inhibition of p97 function in these experiments. Therefore, further experiments will hopefully clarify the relevance of p97 function for Golgi reformation in vivo.

Lastly, it will be important to understand the potential link between the NSF and p97 pathways. Work on the ubiquitin-like modifier SUMO in unrelated processes revealed that it can cross-talk with the ubiquitin system [134]. It is therefore not unlikely that the NSF and p97 pathways may converge on a GATE-16 dependent step, particularly since it has not been excluded that GATE-16 may also modify proteins.

The recent implication of members of the ubiquitin family in Golgi reassembly has revealed new twists in this process, but has also raised many new questions. As always, this already indicates that progress has been made that may finally help us to understand the process.

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