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p97/p47-Mediated Biogenesis of Golgi and ER

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In mammalian cells, the Golgi apparatus and endoplasmic reticulum have typical structures during interphase: stacked cisternae located adjacent to the nucleus and a network of interconnected tubules throughout the cytoplasm, respectively. At mitosis their architectures disappear and are reassembled in daughter cells. p97, an AAA-ATPase, mediates membrane fusion and is required for reassembly of these organelles. In the p97-mediated membrane fusion, p47 was identified as an essential cofactor, through which p97 binds to a SNARE, syntaxin5. A second essential cofactor, VCIP135, was identified as a p97/p47/syntaxin5-interacting protein. Several lines of recent evidence suggest that ubiquitination may be implicated in the p97/p47 pathway; p47 binds to monoubiquitinated proteins and VCIP135 shows a deubiquitinating activity *in vitro*. For the cell-cycle regulation of the p97/p47 pathway, it has been reported that the localization and phosphorylation-dephosphorylation of p47 are crucial. In this review, we describe the components involved in the p97-mediated membrane fusion and discuss the regulation of the fusion pathway.

Key words: membrane fusion, phosphorylation, ubiquitin, VCP.

The endoplasmic reticulum (ER) and Golgi apparatus are key organelles for cellular life. Proteins are synthesized in the ER and transported to the Golgi, where they receive several necessary modifications. The Golgi apparatus dispatches them toward their correct downstream locations. Both the ER and Golgi have unique architectures; the ER is a network structure and the Golgi a stacked structure of flattened membrane disks.

The Golgi apparatus undergoes dramatic transformation during the cell cycle (*1*) (see Fig. 1). During mitosis, it is fragmented into thousands of vesicles and short tubules that are dispersed throughout the cytoplasm. Some or all of them might be absorbed into the ER, although this is a matter of controversy (*2–5*). Lippincott-Schwartz and colleagues studied what happens in the Golgi during mitosis by labeling Golgi enzymes with green fluorescent protein. They showed these proteins fled to the ER during mitosis and re-emerged from the ER to the Golgi at the end of mitosis. It was concluded that all Golgi components were absorbed into the ER during mitosis and the Golgi were rebuilt from the ER after cell division (*4*). On the other hand, Warren and colleagues investigated the transport of Golgi matrix proteins in interphase and proposed that the Golgi might be an autonomous organelle rather than one in simple dynamic equilibrum with the ER (*5*). They also examined the fate of the Golgi membrane during mitosis using time-lapse fluorescence microscopy, EM reconstruction of mitotic cells from serial thin sections, and cytochemical staining using a HRP-Golgi resident chimera (*2*). No evidence for Golgi residents in the ER during mitosis was found and, hence, they argued that the Golgi apparatus partitioned itself at mitosis without being absorbed into the ER. Recently, Malhotra's group devised a procedure to address whether Golgi membranes fuse with the ER during mitosis, which rests on the detection of interactions between ER and Golgi proteins using coexpression of an FKBP-tagged Golgi enzyme with an ER-retained protein fused to FRAP (*6*) . After the doubly transfected cells had progressed through mitosis in the presence of rapamycin, no Golgi protein was found in the ER. They thus concluded that Golgi membranes remain separate from the ER during mitosis in mammalian cells.

At telophase, the mother cell divides into two daughter cells, and a Golgi apparatus is rapidly reassembled from fragments within each daughter cell (*7*). Experiments using an *in vitro* Golgi reassembly assay showed that reassembly from membrane fragments requires at least two ATPases: *N*-ethylmaleimide–sensitive factor (NSF) and p97 (also known as VCP) (*8*, *9*). It is unclear why the two distinct pathways are necessary for the reassembly of Golgi, although it has been speculated that the NSF and p97 pathways may be required for heterotypic and homotypic membrane fusion, respectively (*10*). In contrast to the Golgi, the ER network is not completely fragmented during mitosis (*11*), and ER membrane fusion requires the p97 pathway but not the NSF pathway.

Essential factors for p97-mediated membrane fusion

The mechanism of NSF-mediated membrane fusion has been well characterized. NSF and soluble NSF attachment protein (SNAP) are ubiquitous components of NSF-driven membrane fusion. How is accurate membrane fusion controlled in the NSF pathway? The specificity of the membrane fusion is determined by at least two distinct mechanisms, vesicle tethering and SNAP recep-

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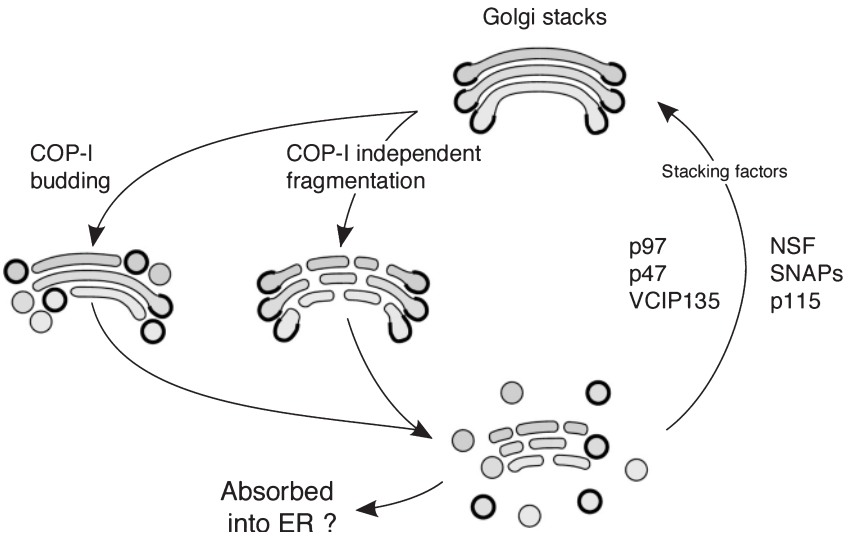

Fig. 1. **The cell cycle-dependent change of the Golgi apparatus.** Once a cell enters mitosis, Golgi stacks are disassembled into small vesicles and tubules. After cell division, the Golgi apparatus is reassembled from fragments in a daughter cell.

tor (SNARE)–mediated docking (*12–15*). The SNARE-mediated docking is preceded by the tethering of vesicles to their target membrane. The tethering restricts vesicle movement to a distinct range and promotes SNARE complex assembly. SNAREs are the key components of the fusion machinery. They are membrane-associated proteins and characterized in their sequence and structure by the SNARE motif, which is a coiled-coli structure with a central ionic layer (*16*, *17*). When v-SNAREs on a transport vesicle pair with their cognate t-SNAREs on a target membrane, forming SNAREpins, the two lipid bilayers are brought into close apposition and fusion occurs. The SNARE complex resulting from the membrane fusion is disassembled by NSF. NSF binds to SNAREs *via* SNAP and dissociates the SNARE complex using ATP hydrolysis so that SNAREs can be recycled for further fusion (*18*, *19*).

In contrast to NSF, much less is known about the mechanism of action of p97. Only two essential cofactors in p97-mediated fusion pathway have so far been identified, namely p47 and VCIP135 (VCP[p97]/p47 complex-interacting protein, p135). Syntaxin5 (syn5) is only known SNARE involved in the p97 pathway.

p97/VCP

p97/VCP, known as Cdc48 in yeast, is an AAA ATPase. It is widely distributed and very abundant. It is barrel-shaped and consists of homohexamer of a 97 kDa protein. p97/VCP/Cdc48 has been reported to be involved in several events: the retro-translocation of unfolded proteins from the endplasmic reticulum (ER), ubiquitin-proteasome dependent proteolysis, spindle disassembly and membrane fusion. p97/VCP/Cdc48 is thought to have several adaptors for its distinct functions (see Table 1).

Like NSF, p97 contains two ATPase domains (D1 and D2) (*20*). In case of p97, D1 is important for the hexamer formation whereas D2 is required for its function (*21*). Its N-terminus is important for its binding to adaptors. The crystal structure of p97 has been already clarified (*22*).

p47

p47 was first found as an essential factor for the p97-mediated membrane fusion pathway (*23*). It forms a trimer and a tight complex with p97. This complex is essential for the mitotic Golgi reassembly. The addition of p97/p47 complex is required for Golgi reassembly from mitotic Golgi fragments in an *in vitro* function assay, and the addition of p97 alone is not sufficient. p47 is shown to be involved in the reassembly of the Golgi and the ER at the end of mitosis by the microinjection of anti-p47 antibodies into mitotic cells.

p47 has two p97-binding sites in its C-terminus, one of which contains the UBX domain. It also has a UBA domain at its N-terminus, which binds to mono-ubiquitin conjugate and is important for Golgi reassembly (*24*). It is phosphorylated by Cdc2 kinase during mitosis (*25*).

VCIP135

VCIP135 (VCP[p97]/p47 complex–interacting protein, p135) is a second essential factor for p97/p47-mediated membrane fusion pathway (*26*). It has a UBX domain,

Table 1. **Adaptor proteins that interact with p97/VCP/Ccd48.**

Function	Adaptor	
Golgi reassembly	p47	(*23*)
	VCIP135	(*26*)
ER network formation	p47	(*26*)
	VCIP135	(*26*)
tER formation	p47	(*29*)
Nuclear envelope reassemble	Ufd1/Npl4	(*39*)
	p47	(*39*)
Proteasomal degradation	Ufd2	(*40*) (*41*)
ER-associated degradation	Ufd1/Npl4	(*42*) (*43*)
ubiquitin-dependent processing	Ufd1/Npl4	(*44*) (*45*)
DNA replication?	DUF	(*46*)
Spindle disassembly	Ufd1/Npl4	(*47*)
Vacuole formation in cells containing polyQs	?	(*48*)
ER function?	SVIP	(*49*)

which is important to its binding to p97. VCIP135 also binds to the p97/p47/syntaxin5 complex and dissociates it *via* p97-catalyzed ATP hydrolysis. In addition, the microinjection of anti-VCIP135 antibodies into lining cells showed that VCIP135 is required for Golgi assembly and ER network formation *in vivo* (*26*). Recently, VCIP135 is reported to have deubiquitinating activity, which is necessary for Golgi reassembly *in vitro* (*27*).

SNARE

Syntaxin5 (syn5) is a receptor protein for p97/p47-mediated Golgi reassembly. p47 mediates the binding of p97 to syn5. Syn5 is also utilized in NSF-mediated mitotic Golgi reassembly. p47 and alpha-SNAP, an adaptor of NSF, compete with each other for the binding to syn5 (*28*). Syn5 is so far the only SNARE reported in the p97/p47 pathway. Although syn5 has a tendency to polymerize, several structural studies predict that it is difficult to form a SNAREpin without the assistance of other SNAREs or SNARE-like proteins.

Syn5 is also required for the fusion of transitional ER membranes (*29*). Ufe1p is reported to be a SNARE for the ER membrane fusion in yeast (*30*) and its homologue in mammals is syntaxin18 (*31*). However, there is so far no data showing that syntaxin18 is a SNARE for p97/p47-mediated fusion.

Tethering

An important difference between the p97/p47 and NSF pathways of Golgi reassembly is that p115, a vesicle tethering protein, is needed for the NSF but not the p97/p47 pathway (*10*). p115 acts to tether vesicles to membranes using GM130 and Giantin as the membrane-bound receptors (*32*). This is not to say that the p97/p47 pathway does not need the membranes to be tethered before fusion can occur. It is just that the tethers have to be membrane-bound rather than soluble. They also have to be tightly membrane-bound, since mitotic Golgi fragments treated with high salt are still able to fuse in the presence of p97/p47 and VCIP135 alone (*26*).

Cell-cycle regulation of the p97/p47 pathway

The phosphorylation was reported to be important for the disassembly of the Golgi apparatus, but the debate continues as to which kinases are responsible. Suggested kinases include Cdc2 kinase (*33*, *34*), mitogen-activated protein (MAP) kinase kinase (*34*, *35*), and Polo-like kinase1 (*36*). Since the Golgi apparatus has a complicated structure, its disassembly is thought to occur in several steps. These kinases might be involved in these disassembly steps. Malhotra and colleagues proposed two sequential fragmentation reactions during mitosis in mammalian cells (*37*). First, PlK1 and MEK1 are required, and the pericentriolar Golgi apparatus is converted into tubulo-reticular elements (Golgi blobs). These Golgi blobs undergo further disassembly into small vesicles through the action of Cdc2 kinase.

Golgi disassembly–reassembly requires blocking of membrane fusion at early mitosis and its unblocking at late mitosis (*1*). NSF binds to SNAREs via α-SNAP and activates them for membrane fusion (*18*). NSF needs the assistance of p115-GM130 tethering (*32*). At early mitosis, GM130 is phosphorylated on Serine-25 by Cdc2 kinase. This phosphorylation disrupts the tethering of p115-GM130, resulting in the mitotic inhibition of the NSF pathway (*33*). In contrast to NSF, p97 does not require p115-GM130 tethering (*9*). p47 is phosphorylated on Serine-140 by Cdc2 at mitosis (*25*). The phosphorylated p47 does not bind to Golgi membranes. An *in vitro* assay shows that this phosphorylation is required for Golgi disassembly. p47 has nuclear localization signals and localizes to the nucleus at interphase. Hence, cell-cycle regulation of the p97/p47 pathway is thought to occur as follows. Once the cell enters mitosis, its nuclear envelope is broken down, and p47 enters the cytoplasm. At the same time, p47 is phosphorylated by mitotically activated Cdc2 kinase. As the phosphorylated p47 cannot bind to Golgi membranes, p97/p47-mediated membrane fusion is inhibited, resulting in the fragmentation of Golgi membranes. At late mitosis, p47 is dephosphorylated and binds to Golgi membranes. This allows p97/p47-mediated membrane fusion to reassemble the Golgi apparatus. When nuclear envelopes are formed in daughter cells, p47 moves to the nucleus and the p97/p47 pathway is suppressed. Accordingly, the membrane fusion mediated by p97/p47 functions is for the reassembly of organelles at the end of mitosis, but not for their maintenance during interphase. The next question is whether the fragmentation of Golgi at mitosis is prevented by keeping p97/p47-mediated fusion activity.

p47(S140A), which cannot be phosphorylated, was microinjected into prophase (or early prometaphase) cells and their Golgi structures were investigated (*25*). In the cells injected with p47(S140A), the dispersion of Golgi membranes was mostly suppressed and Golgi clusters showing bright staining were observed through all mitotic phases. The observation using EM showed that the injection of p47(S140A) allowed the cells to keep Golgi stacks during mitosis. Interestingly, the p47(S140A)-injected cells achieved equal partitioning of Golgi membranes into daughter cells. This means that Golgi fragmentation-dispersion may not be obligatory for equal partitioning even in mammalian cells. These results have raised another two major questions. How is equal partitioning achieved? Why is Golgi fragmentation necessary at mitosis? For the second question, the interesting observation that Golgi fragmentation is necessary for entry into mitosis is presented by Malhotra and coworkers (*38*). Microinjection of anti-GRASP65 antibodies or a GRASP65 fragment into cells inhibited Golgi fragmentation and prevented the cells from entering into mitosis.

Regulation of p97/p47-mediated membrane fusion by ubiquitination and deubiquitination

p47 has a UBA domain at its N-terminus and binds to monoubiquitin, not to polyubiquitin (*24*). Warren's group has recently reported that VCIP135 shows a deubiquitinating activity and that its activity is important for p97/p47-mediated Golgi reassembly (*27*). Thus, ubiquitination-deubiquitination of some factor is thought to play an important role in the p97/p47 pathway. However, it is likely that the ubiquitination is not essential for p97/p47-medated fusion but it is necessary for its regulation. A p47 mutant which lacks a UBA domain never binds to ubiquitin, but it still has almost 30% of membrane fusion

activity compared with p47wt (*24*). Considering the specificity of the binding between p47 and the ubiquitinated protein, p47 may recognize a specific domain in the target protein and the interaction between ubiquitin and p47 may work to increase the binding affinity.

It has been reported that the p97/p47 pathway works for reassembly of the Golgi at the end of mitosis, not for Golgi maintenance during interphase (*25*). The microinjection of anti-p47 antibodies inhibited the Golgi reassembly at the end of mitosis, while it had no effect on Golgi maintenance during interphase. Some p47 is still localized to the cytoplasm together with p97 and VCIP135; nevertheless the p97/p47 pathway never works during interphase. One possible explanation is that ubiquitin might be conjugated to the factor(s) only during mitosis. The role of ubiquitination in the p97/p47-mediated membrane fusion is entirely unclear, and the identification of a target protein for ubiquitination is necessary to clarify the mechanism of the p97/p47 pathway.

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