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Yeast Golgi apparatus – dynamics and sorting

A. Nakano

Molecular Membrane Biology Lab., RIKEN Discovery Research Institute, 2-1 Hirosawa, Wako, Saitama 351-0198 (Japan), Fax: +81 48 462 4679, e-mail: nakano@riken.jp

Abstract. The yeast *Saccharomyces cerevisiae* is one of the best-studied organisms to understand molecular mechanisms of membrane traffic, but as far as the organization of the Golgi apparatus is concerned, yeast is only just beginning to yield clues about how dynamic and flexible the organelle is.

Key words. Golgi stacks; dispersed Golgi; membrane recycling; cisternal maturation.

Introduction

In this issue composed of a number of reviews, it may appear unnecessary to introduce to readers what the Golgi apparatus looks like and what its functions are. However, if you think about the Golgi in budding yeast, one of the most popular organisms in which to study protein trafficking, you may be surprised to find that it does not behave as you might imagine. It is because the image of the Golgi that most people have in their mind is perhaps not the general feature of this organelle. I will try to describe in this short review the essence of Golgi dynamics and its role in protein sorting, which we can learn from yeast.

Classic image

The general image of the Golgi from textbooks is that of stacks of flat cisternae. The number of cisternae in a single stack varies, but in typical animal cells it seems to be around seven [1]. Those who are working on mammalian cells will also say that the Golgi complex is clustered in the perinuclear region of the cell. This is believed to be due to the presence of the microtubule-organizing center in this region. However, it should be remembered that such clustering of the Golgi is a feature developed only in higher animals. Plant and yeast cells do not have clustered Golgi but let them scatter throughout the cytoplasm. Such a dispersed pattern of Golgi is also seen in invertebrates such as *Drosophila*. To understand why the Golgi is clustered in higher animals, study of the sea urchin [2] is very intriguing. During the early development of sea urchin embryos, the Golgi is scattered until the ninth cell division. At this stage, localization of Golgi suddenly changes to the clustered pattern. At this moment, the blastula embryo forms cilia on its surface and secretes the hatching enzyme. It may be explained that a prerequisite for the establishment of strongly polarized secretion is clustering of the Golgi complex.

In the case of the yeast *Saccharomyces cerevisiae*, what is more surprising is that the Golgi cisternae are not even stacked. It is very rare to observe multiple layers of Golgi cisternae in *Saccharomyces cerevisiae* by electron micrography [3]. Accordingly, early and late cisternae usually do not overlap by double labeling (see fig. 1). It is not clear, however, whether this is a general feature of various yeast species. *Schizosaccharomyces pombe* and *Pichia pastoris* appear to have more organized Golgi layers, but there is an argument as to whether they are indeed like stacks of animal and plant cells [4]. In any case, if *Saccharomyces* yeast cells do not require stacked Golgi cisternae, why do others? What is the advantage of cisternal stacking? The answer is not obvious.

Golgi as the sorting center

It is often said that the most important function of the Golgi apparatus is its role as the sorting center of membrane traffic. For example, it is placed at the branching point of the secretory pathway and the lysosomal/vacuolar pathway. The *trans*-Golgi network and late endosomes may be involved in merging of the lysosomal/vacuolar pathway and the endocytic pathway. Even for localization

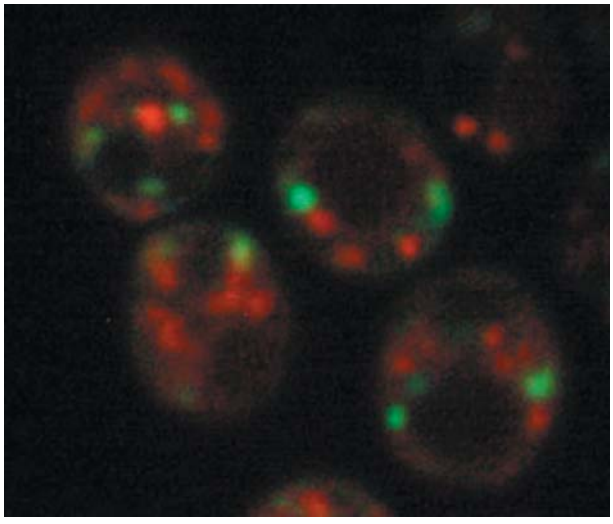

Figure 1. Dual imaging of yeast Golgi. The early Golgi marker GFP-Rer1p and the late Golgi marker mRFP-Gos1p are simultaneously expressed and observed using a confocal laser scanning microscope. Discrete fluorescence of green and red structure is obvious.

of endoplasmic reticulum (ER) resident proteins, the Golgi plays an essential role by sending them back via the Golgi-to-ER retrieval mechanism [5].

The Golgi apparatus also appears to be important for establishing divergent secretory pathways. Constitutive secretion versus regulated secretion and apical secretion versus basolateral secretion are good examples of diversity within the secretory pathway. Although yeast is a very simple microorganism, it has contributed much to our understanding of such sorting functions of the Golgi. Below is a summary of what we have learned from yeast.

Sorting in ER-Golgi transport

I do not intend to review the enormous number of studies on how proteins are sorted and transported between the ER and the Golgi. There are still arguments, but let me simply state the widely accepted view that anterograde ER-to-Golgi transport is mediated by COPII vesicles, and the opposite traffic – retrograde Golgi-to-ER transport – is mediated by COPI vesicles. For formation of COPII vesicles from the ER membrane, activation of Sar1p, a small GTPase, by Sec12p, the Sar1p-specific guanine nucleotide exchange factor, is essential [5]. Similarly, COPI vesicles are formed by activation of the Arf1 GTPase on the Golgi membrane.

The concept of ER retention was clearly revealed upon the discovery of the KDEL signal [6], but later on, ER localization of KDEL/HDEL-harboring proteins was demonstrated to be achieved by recycling between the ER and the Golgi [7, 8]. The receptor for the KDEL/HDEL signal (called Erd2p in yeast) is located in the Golgi, and it captures any processing molecules that escape from the ER and sends them back to the organelle [9].

We also demonstrated that Sec12p, is an ER resident membrane protein, also recycles between the ER and the Golgi [10, 11]. By genetic screening, we identified two yeast genes, *RER1* and *RER2*, which are involved in the ER localization of Sec12p [11]. Rer1p has now been demonstrated to be the sorting receptor for a set of ER membrane proteins including Sec12p and Sec71p [12–16] (fig. 2). It is again located in the Golgi and retrieves those ER proteins back to the ER. Like Erd2p, the Golgi localization of Rer1p is dynamically attained. It also recycles between the Golgi and the ER, and is mislocated to the vacuole if its Golgi localization signals or COPI components are mutated. The dynamic equilibrium of Golgi resident proteins is one of the most important features of this organelle.

Two different mechanisms appear to operate in the location of Sec12p to the ER. One is the Rer1p-dependent retrieval from the Golgi, and the other appears to be static retention in the ER. We identified two distinct structural domains of Sec12p that are important for its ER localization [13]. The retrieval signal was contained in the transmembrane domain (TMD) of Sec12p as at least two polar amino acid residues that are separated by a certain length of stretch of hydrophobic residues. A very similar motif is also found in Sec71p, whose membrane topology is completely opposite to that of Sec12p. The modes of interaction between these different classes of ligand and Rer1p have been analyzed [16].

The structural information in Sec12p for static retention in the ER is confined to the N-terminal cytoplasmic domain. However, as this catalytic domain elaborates a very

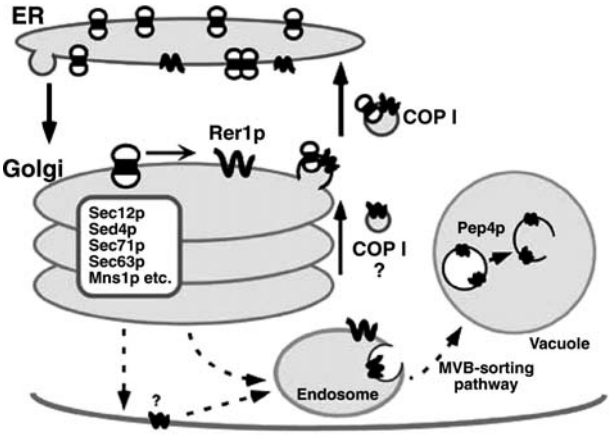

Figure 2. Dynamic function of Rer1p. Rer1p recognizes the signal in the TMD of cargo proteins, and actively recycles between the early Golgi and the ER. When it is mislocalized to the late Golgi, it is retrieved to the early Golgi by the COPI vesicles (arrows). In COPI mutants, Rer1p is no longer able to localize to the Golgi and is transported to the vacuole via the MVB sorting pathway (dotted arrows). Adapted from [15].

complex tertiary structure [17 and our unpublished data], the details of molecular mechanisms remain unclear. In the hope of identifying possible players of ER retention, we analyzed the *RER2* gene and surprisingly found that it encodes *cis*-prenyltransferase, the key enzyme of dolichol synthesis [18, 19]. Dolichol sugars are essential intermediates for N- and O-linked glycosylation in yeast, and *rer2* mutants are defective in the synthesis of glycoproteins and show a variety of phenotypes. However, the defect in correct localization of ER proteins is not a general property of glycosylation mutants of yeast. From these observations, we have suggested that dolichol may have as yet unknown physiological functions in the ER membrane, whose deficiency leads to the malfunction of the ER membrane in protein retention.

While ER resident proteins are left behind in the ER, cargo molecules destined to the Golgi are known to be concentrated and packaged into the forming COPII vesicles. Signals for this anterograde transport have been found on some cargo molecules [20–22]. To enable such positive sorting during vesicle budding, receptor molecules must exist. In a search to identify such receptors, Erv29p was recently demonstrated to be a cargo receptor for soluble proteins such as the glycosylated α-factor precursor [23]. We focused our attention on Emp47p, a yeast homologue of ERGIC-53, which was reported to act as a cargo receptor for glycoproteins in mammalian cells [24, 25]. We found another homologue of ERGIC-53 in the yeast genome, named Emp46p, which is 45% identical to Emp47p [26]. They are both localized to the Golgi at the steady state and recycle between the Golgi and the ER. The yeast cells disrupted for both Emp46p and Emp47s show a defect in secreting a set of glycoproteins, suggesting that they also function as glycoprotein cargo receptors. In an attempt to identify cargo molecules by cross-linking, we discovered that Emp46p and Emp47p form a large heterooligomer complex [27]. This oligomerization is important for exit from the ER. Emp47p can get out of the ER by itself if it forms a homooligomer. Emp46p, in contrast, requires binding with Emp47p to be packaged into COPII vesicles. This can be interpreted as Emp46p needing Emp47p as a cargo receptor for ER exit. Interestingly, the complex formation is observed both in the ER and in the COPII vesicles but not in the Golgi.

These observations raise a very interesting question. How do sorting receptors recognize the donor and the acceptor compartments? In the case of retrieval receptors such as Erd2p and Rer1p, they must bind to ER proteins in the Golgi and release them in the ER. The situation is completely opposite for the ER-to-Golgi cargo receptors. The milieu of the lumen of these organelles, such as pH and Ca^{2+} concentration, and the status of oligomerization have been thought to explain this puzzling problem, but so far there is no clear evidence. Our observation on differential interaction between Emp46p and Emp47p in the ER and the Golgi may provide us with a valuable clue to answering this question.

Post-Golgi sorting

Protein sorting in and post the yeast Golgi apparatus is a much more complex issue. There are at least two pathways from the Golgi to the vacuole. One is via late endosomes, and the other is a shortcut mediated by the AP-3 adaptor complex [28]. Recent evidence indicates that even the route to the plasma membrane is not a single pathway [29]. The role of ubiquitination is one of the hottest topics in sorting along the endocytic and vacuolar pathways [30]. In late endosomes, some membrane proteins are sequestered from the limiting membrane into internal vesicles, a process called multivesicular body (MVB) sorting, and many molecular components are involved in this MVB sorting pathway [31–35].

Consider one example from our studies, in which post-Golgi sorting by ubiquitination is tightly coupled to the sorting by lipid rafts. It is known that yeast cells somehow sense the nutrient conditions in the surrounding environment. For instance, they can tell the deficiency of particular nitrogen sources in the medium and mobilize the general amino acid permease to the cell surface [36]. Similarly, we realized that the high-affinity tryptophan permease Tat2p resides intracellularly in the rich medium, but gets expressed on the plasma membrane when the tryptophan concentration is lowered [37]. The major intracellular location of Tat2p on rich tryptophan is in late endosomes. Upon the shift from high to low tryptophan, routing of Tat2p appears to be switched in early endosomes toward the cell surface. Interestingly, this nutrient-dependent sorting of Tat2p is impaired in *erg6*, a mutant defective in the synthesis of ergosterol [37]. The missorted Tat2p is then delivered to vacuoles and degraded. We also found that this targeting of Tat2p to vacuoles involves the MVB sorting pathway. In wild-type cells, Tat2p stays on the limiting membrane of late endosomes, but in the *erg6* mutant Tat2p enters the MVB pathway and is thus subject to rapid vacuolar degradation. This reminded us of other work on the role of ubiquitin in MVB sorting and led us to examine the ubiquitination status of Tat2p. Tat2p is indeed polyubiquitinated in yeast cells, but the extent of ubiquitination appeares to be highly elevated in the *erg6* mutant [37]. The requirement of normal ergosterol synthesis for the correct sorting of Tat2p suggests the involvement of lipid rafts [38]. The presence of detergent-resistant membrane domains in yeast has already been reported [39]. In fact, Tat2p is distributed in the non-raft detergent-soluble fractions on high tryptophan but enters the detergent-resistant raft fraction upon shift to low tryptophan [37]. This segrega-

tion into the raft fraction was again impaired in the *erg6* mutant. Surprisingly, the knockout of Bul1p, a subunit of the Rsp5 ubiquitin ligase complex, resulted in the almost complete recovery of the Tat2p sorting, indicating intimate relationship between the ubiquitin-dependent sorting and the raft sorting (fig. 3).

Inter-cisternal transport

The mechanism of inter-cisternal transport is a very controversial issue [40]. It is not intended to repeat the debate between the vesicular transport versus the cisternal maturation model here, but just to remind readers that early and late compartments of yeast Golgi seem to exist separately. Transport between these compartments must be much easier to observe than in higher organisms that develop the stack structure. We have begun to approach this problem by a new confocal imaging technique, described below.

Real-time imaging of the yeast secretory pathway

The discovery of green fluorescent protein brought us a revolution in cell biology: live cell imaging. With the use of confocal laser scanning microscopy, we can now easily observe the dynamics of organelles in living cells. However, there are several limitations in the currently available microscopic systems, such as speed and sensitivity. Most of the commercial confocal laser microscopes utilize the galvanomirror method for beam scanning, which requires seconds to obtain a high-resolution image. Even with a high-speed setting at the expense of resolution, normal CCD (charge-coupled device) cameras are not sensitive enough to detect weak fluorescence in the subsecond exposures, that are required for transport vesicle imaging.

To overcome these difficulties, we recently started a project to develop a new microscope system that enables observation of membrane-bound structures as small as transport vesicles (i.e. less than 100 nm in diameter), which move around in the cell extremely rapidly. In collaboration with Yokogawa Electric Corporation and NHK (Japan Broadcasting Corporation), we have set up a high-speed (30 frames/s) and ultra-high-sensitivity confocal system using a spinning-disk Nipkow scanner and a HARP (high-gain avalanche rushing amorphous photoconductor) camera [41]. Movies are accessible at the web site http://www.jstage.jst.go.jp/article/csf/27/5/27_349/_applist where you can view rapid movements of COPI and COPII vesicles visualized through GFP-Rer1p expression. We are now in the process of developing the system further toward rapid simultaneous observation of multiple fluorescent markers. An example of yeast cells expressing GFP-Rer1p (an early Golgi marker) and RFP-Gos1p (a late Golgi marker) is shown in figure 1. This cutting-edge technology will let us challenge the many problems that

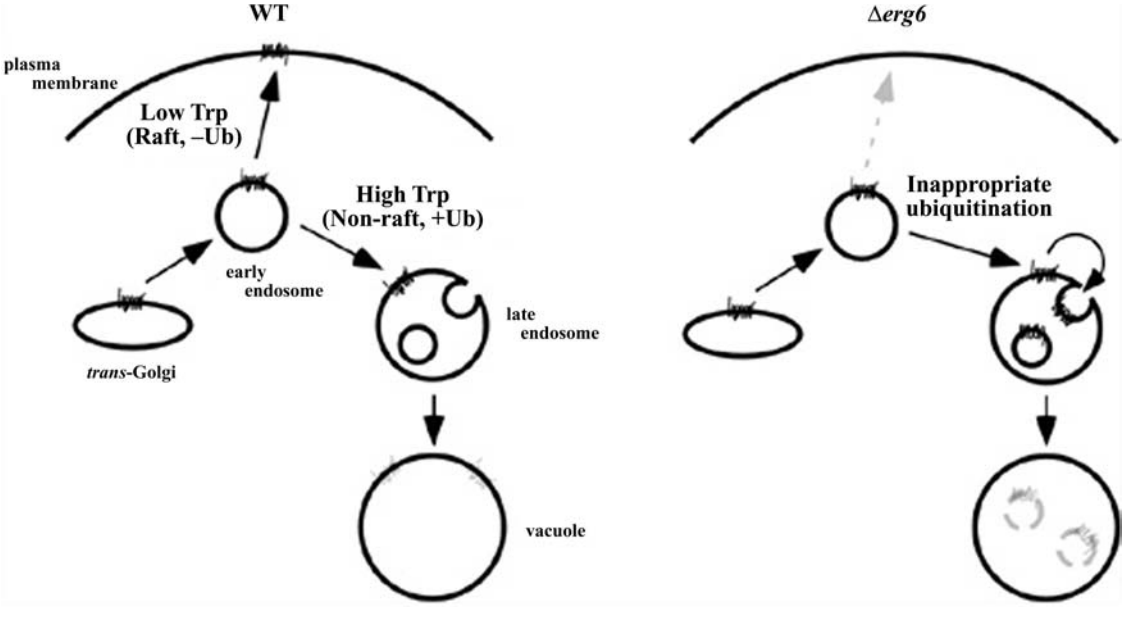

Figure 3. Targeting of Tat2p in wild-type and Δ*erg6* cells. In wild-type (WT) cells, plasma membrane delivery of Tat2p is controlled by the external tryptophan concentration. The sorting likely occurs in early endosomes. Polyubiquitin acts as a sorting signal to the non-raft, vacuolar trafficking pathway, whereas raft association is required for plasma membrane targeting. In Δ*erg6* cells, Tat2p is sequentially missorted in early and late endosomes. These defects were suppressed by inhibition of ubiquitination, suggesting that Tat2p is inappropriately ubiquitinated in Δ*erg6*. Adapted from [37].

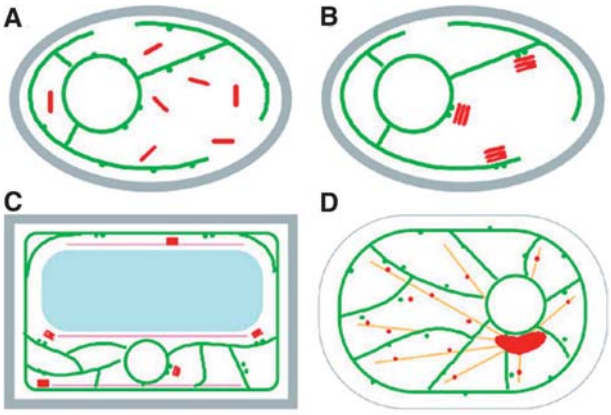

Figure 4. Organization of Golgi apparatus in different types of cells. (*A*) *S. cerevisiae*; (*B*) *P. pastoris*; (*C*) higher plant cell; (*D*) mammalian cell. Green, ER; red, Golgi; pink, actin filaments; orange, microtubules.

will be solved by observing live material. The big controversy of intra-Golgi transport may well be settled if multiple markers are visually chased in living cells.

Other problems and perspectives

There are still many other problems that yeast Golgi can help answer. For instance, how do cells inherit Golgi complexes? If the cisternal maturation model is correct, how are the resident components retrieved from later to earlier compartments? Is the new *cis* cisterna synthesized de novo? To address these questions, another species of yeast, *Pichia pastoris*, offers a wonderful experimental system. *P. pastoris* cells have a fewer number of Golgi complexes than *S. cerevisiae*, and these appear to be located in the close vicinity of the transitional ER, the budding sites of COPII vesicles [42]. Evidence has been presented that the Golgi apparatus indeed forms de novo in *P. pastoris* [43, 44]. It is of interest to find what controls the difference between the *S. cerevisiae* and *P. pastoris* Golgi apparatus. A candidate to determine the pattern of the transitional ER, dispersed in *S. cerevisiae* and discrete in *P. pastoris*, is Sec12p [42, 43]. The comparison of the patterns of the transitional ER and the Golgi in *S. cerevisiae*, *P. pastoris*, mammalian and plant cells is shown in figure 4 (partly adapted from [41]). This will serve as a reminder of how differently the ER and Golgi are organized in cells from different organisms. Such diversity of the Golgi indicates that the fundamental nature of this organelle is dynamic and flexible. The evolutionary and developmental differentiation of the Golgi will be an exciting topic to pursue.

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