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LETTERS

Live imaging of yeast Golgi cisternal maturation

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There is a debate over how protein trafficking is performed through the Golgi apparatus[1–4]. In the secretory pathway, secretory proteins that are synthesized in the endoplasmic reticulum enter the early compartment of the Golgi apparatus called *cis* cisternae, undergo various modifications and processing, and then leave for the plasma membrane from the late (*trans*) cisternae. The cargo proteins must traverse the Golgi apparatus in the *cis*-to-*trans* direction. Two typical models propose either vesicular transport or cisternal progression and maturation for this process. The vesicular transport model predicts that Golgi cisternae are distinct stable compartments connected by vesicular traffic, whereas the cisternal maturation model predicts that cisternae are transient structures that form *de novo*, mature from *cis* to *trans*, and then dissipate. Technical progress in live-cell imaging has long been awaited to address this problem. Here we show, by the use of high-speed three-dimensional confocal microscopy, that yeast Golgi cisternae do change the distribution of resident membrane proteins from the *cis* nature to the *trans* over time, as proposed by the maturation model, in a very dynamic way.

We have been developing a high-performance confocal laser scanning microscopic system, by combining the spinning-disk (Nipkow disk) confocal scanning method and HARP (high-gain avalanche rushing amorphous photoconductor) cameras[5]. The most recent prototype features multi-colour observation with extremely high sensitivity and high signal-to-noise ratio, permitting the high-speed observation of weak fluorescent signals within a living cell. We applied this technology to the observation of the Golgi apparatus in yeast cells.

The yeast *Saccharomyces cerevisiae* has a unique feature in the organization of its Golgi. Unlike higher animal and plant cells, in which the Golgi apparatus forms a stacked structure of several cisternae, *S. cerevisiae* usually shows single layers of Golgi cisterna scattering in the cytoplasm[6–8]. The clustering of the Golgi in the perinuclear region, seen in mammalian cells, does not take place in yeast cells; yeast therefore provides an ideal system in which to view individual Golgi cisternae under a microscope[9].

Figure 1 shows the yeast cells simultaneously expressing two Golgi markers with different fluorescence colours as revealed by a confocal microscope. In Fig. 1a, Rer1, a retrieval receptor for membrane proteins of the endoplasmic reticulum[10,11], was fused to the green fluorescent protein (GFP) and used as a *cis*-Golgi marker. Gos1 is a SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) molecule in a later compartment of yeast Golgi[12,13], and was used as a medial marker in the form of a fusion with the monomeric red fluorescent protein (mRFP)[14]. In Fig. 1b, Sed5, another SNARE molecule residing mainly in the *cis*-Golgi[15,16], was fused to mRFP, and Sec7, a guanine-nucleotide exchange factor for Arf GTPases in the *trans*-Golgi[17,18], was fused to GFP. These fusion proteins were selected out of many constructs we had made, because they proved fully functional by complementation of mutant phenotypes without affecting the morphology of the Golgi apparatus. In either of the two combinations, green and red signals were mostly discrete, confirming that early and late Golgi cisternae scatter separately in the yeast cytoplasm. Effects of traffic mutations on

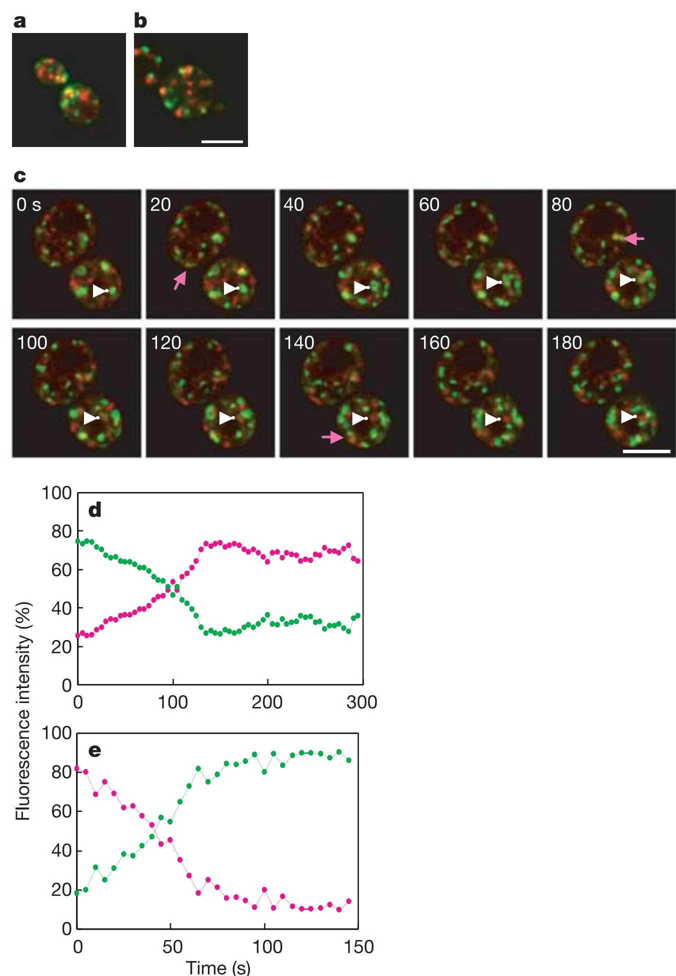

Figure 1 | Dual-fluorescence observation of yeast Golgi cisternae. **a**, Wild-type cells expressing GFP–Rer1 (*cis*, green) and mRFP–Gos1 (medial, red). **b**, Wild-type cells expressing mRFP–Sed5 (*cis*, red) and Sec7–GFP (*trans*, green). **c**, Cells as in **a** were subjected to sequential recording at the rate of one frame per 5 s (see Supplementary movie 1). Frames selected at intervals of 20 s are shown. **d**, Analysis of colour change of the cisterna marked by the white arrowheads in **c**. Relative fluorescence intensities in the green and red channels (points coloured accordingly) were normalized to the total and are plotted against time. **e**, Similar analysis of colour change from the experiment using cells as in **b**. Scale bars, 5 μm.

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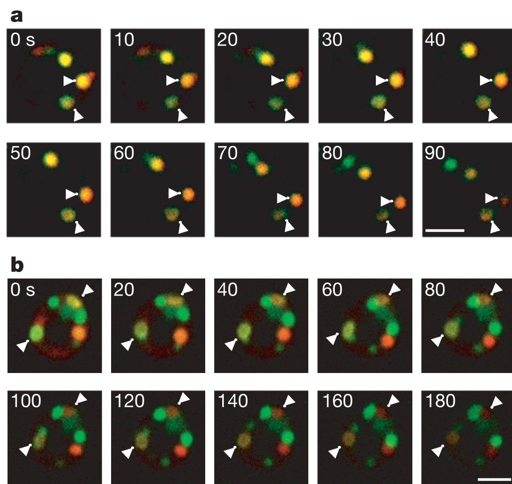

Figure 2 | Dual fluorescence observation with the αCOP mutant *ret1-1*. **a**, *ret1-1* cells expressing GFP–Gos1 (medial, green) and Sec7–mRFP (*trans*, red) were preincubated at the restrictive temperature 37 °C for 30 min and then observed under a microscope whose stage was kept at 37 °C. Images were taken at the rate of one frame per 5 s. Frames at intervals of 10 s are shown. White arrowheads indicate the cisternae whose colour changed. **b**, Similar analysis to that in **a**. Frames at intervals of 20 s are shown. Scale bars, 2 μm. See also Supplementary movies 2 and 3.

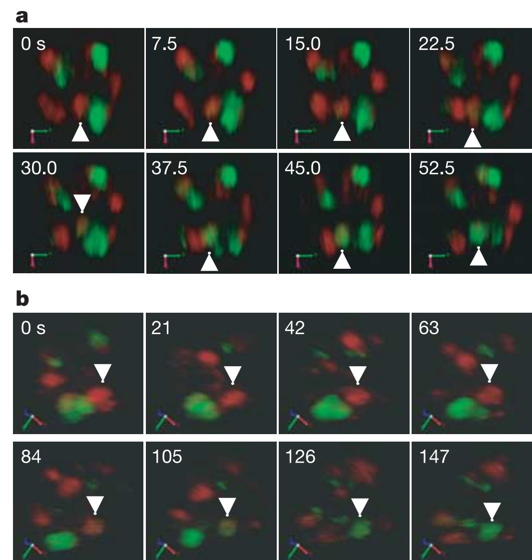

Figure 3 | Three-dimensional observation of yeast Golgi cisternae. Wild-type yeast cells expressing mRFP–Sed5 (*cis*, red) and Sec7–GFP (*trans*, green) were observed under a high-speed confocal microscope with scanning in the *z* axis. **a**, Five optical slices 1 μm apart were taken at 0.5-s intervals to cover a whole cell. Three-dimensional images were reconstructed into a movie (Supplementary movie 4), of which representative views are shown. **b**, As in **a** except that seven optical slices 1 μm apart were taken at 1-s intervals (Supplementary movie 5). Scale bars, 2 μm.

the localizations of these markers are shown in Supplementary Fig. S2.

Next we attempted to follow any changes of individual cisternae in real time. If the classic vesicular transport model is correct, *cis*, medial and *trans* cisternae should be stable compartments, and their fluorescence colours marked by resident proteins should stay unchanged. However, what we observed was a clear change of colour over time. Figure 1c shows a typical example of yeast cells expressing GFP–Rer1 (*cis*, green) and mRFP–Gos1 (medial, red) (see also Supplementary movie 1). In this sequence of 3 min, one cisterna (white arrowheads), which was green at the beginning, changed its colour to yellow and then to red. This indicates that the cisterna, which was occupied by GFP–Rer1 at time zero, became dominated by mRFP–Gos1 during this period. Other examples of colour change of cisternae can also be seen in the same sequence (pink arrows). Figure 1d shows the time course of apparent colour change for the cisterna marked by the white arrowheads, by plotting relative fluorescence intensities observed in the green and red channels of the camera. It took about 2 min for the conversion from the *cis* to the medial property. Conversely, a colour change of red to green was observed for the cells expressing mRFP–Sed5 (*cis*, red) and Sec7–GFP (*trans*, green). A result of similar time-course analysis is shown in Fig. 1e. These colour changes were always unidirectional; the reverse change of colour was never observed. One more example of colour change for the pair of markers GFP–Gos1 (medial) and Sec7–mRFP (*trans*) is shown in Supplementary Fig. S3. In this case the frequency of colour switch was higher than that in Fig. 1c, perhaps because the extent of overlap was larger for these two residents.

We next asked how these membrane components are transferred between cisternae. Cisternal maturation models often postulate retrograde vesicular traffic from later cisternae to earlier ones, which carry back Golgi-resident proteins. A candidate of such vesicular carriers is COPI vesicles. We examined whether a mutation of COPI coat affects the colour change of yeast Golgi. We chose the αCOP mutant *ret1-1*, which was known to be defective in COPI vesicle formation at the restrictive temperature 37 °C (ref. 19). As shown in Fig. 2, the medial cisternae marked with GFP–Gos1 changed colour to red, representing Sec7–mRFP (*trans*) in *ret1-1* cells at the restrictive temperature (see also Supplementary movies 2 and 3). However, the speed of transition seemed significantly lower in the *ret1-1* mutant. To obtain a rough estimate of the transition rates, we measured the red/green fluorescence ratio during the colour conversion and calculated its doubling time. The colour change of cisternae in wild-type cells at 37 °C took a ratio doubling time of 27 ± 7 s (±s.d.; $n = 11$), whereas in *ret1-1* cells at 37 °C it took 92 ± 39 s ($n = 14$). These observations indicate that COPI vesicles are important in the conversion of cisternae but that other mechanism(s) may also operate.

In the settings of our spinning-disk confocal microscope, the thickness of the confocal plane was 1–2 μm; this did not cover the whole yeast cell, whose diameter was about 5 μm. This problem could be resolved by moving the *z* axis of the microscope, collecting multiple confocal images and reconstructing the three-dimensional (3D) architecture. The sequences of pictures in Fig. 3 show whole 3D images of yeast cells expressing mRFP–Sed5 (*cis*, red) and Sec7–GFP (*trans*, green) (Supplementary movies 4 and 5). If necessary, the projected images were appropriately tilted to minimize the overlapping of individual cisternae. Real-time 3D observation of the Golgi cisternae again revealed dynamic features of their conversion. Single isolated cisternae changed their properties over time (white arrowheads).

We also tried to improve the spatial resolution of confocal images. The deconvolution technique was quite powerful in making 3D-reconstructed observations. Two typical results on the cells expressing medial (mRFP–Gos1) and *trans* (Sec7–GFP) markers are shown in Fig. 4 and Supplementary movies 6 and 7. With deconvolution, Golgi cisternae no longer look like flat discs but display complex structures. An enlargement of the zero-time picture of Fig. 4a is shown in Fig. 4b. The Golgi cisternae look mostly fenestrated and are sometimes reminiscent of the tubular network proposed by studies by electron microscopy[7,20,21]. With this improved resolution, the conversion of colours from red to green was even more marked. In the intermediate state of transition there seemed to be a segregation of domains. In the example shown in Fig. 4c, almost all the cisternae observed at the beginning exhibited

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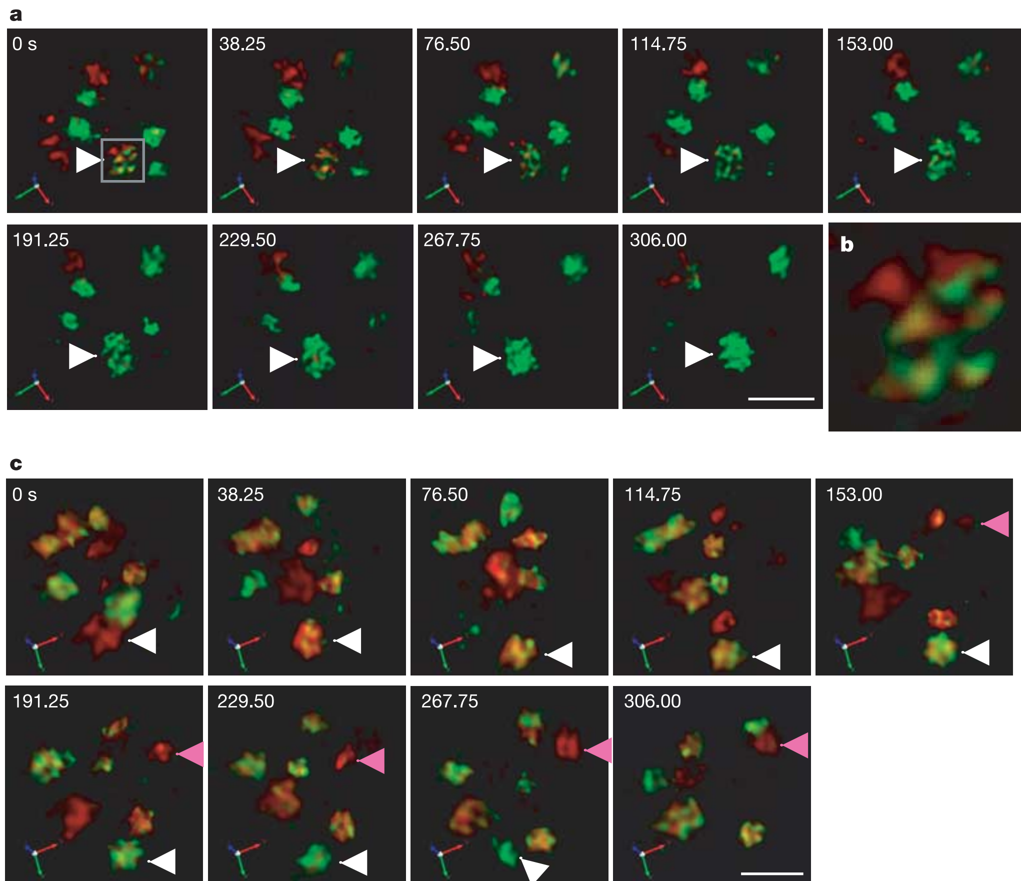

Figure 4 | Three-dimensional deconvolution observation of yeast Golgi cisternae. Wild-type yeast cells expressing mRFP–Gos1 (medial, red) and Sec7–GFP (*trans*, green) were observed under a high-speed confocal microscope. **a**, Fifty optical slices 0.1 μm apart were taken at 0.25-s intervals to cover a whole cell. Three-dimensional images were reconstructed, deconvolved by the parameters optimized for the spinning-disk confocal scanner and organized into a movie (Supplementary movie 6). Representative views of the movies are shown here. **b**, An enlargement of the zero-time picture of sequence **a** (indicated by the white square). **c**, As in **a** except that 50 optical slices 0.14 μm apart were taken (Supplementary movie 7). Scale bars, 2 μm.

the colour conversion within 5 min. The disappearance of green cisternae (white arrowheads) and the new appearance of red cisternae (pink arrowheads) are also clearly visible.

Our data showing that the αCOP mutant slows down but does not completely block the colour conversion of cisternae (Fig. 2) indicate not only important roles for COPI vesicles but also the presence of other mechanisms. For example, tubular connection has recently been shown to form between Golgi cisternae in mammalian cells[22,23]. Indeed, in our experiments (Figs 3 and 4) we often observed the close approach of two distinct cisternae to each other, indicating a possible direct contact between them. Improvement of our imaging methodology should be further pursued to provide an unambiguous understanding of how membrane components are conveyed between cisternae. Quantitative kinetic analysis with a variety of mutants will help us dissect the contributions of different mechanisms.

Our observations, together with a similar study from Glick's group[24], have clearly indicated that Golgi cisternae do mature over time in yeast cells (see Supplementary Fig. S1 for our model). The contribution to the sorting events of membrane segregation during maturation will certainly be an issue to be pursued next. Many more questions remain to be answered. The rate of maturation seems to be fast enough to explain the overall transit of secretory cargo. However, given that the rates of transport may vary from cargo to cargo, it will be necessary to examine how various cargo proteins are travelling in maturing cisternae. The mechanism of unidirectional maturation also needs to be explained. The technology of live-cell imaging is still in progress. The challenge of resolution limits in time and space will certainly be a way of approaching these further exciting problems.

METHODS

Two microscopic settings were used for confocal fluorescence imaging of living cells. For normal two-dimensional observations, an Olympus BX-52 microscope was equipped with a CSU10 spinning-disk confocal scanner (Yokogawa Electric Corporation) and an ORCA-3CCD charge-coupled device colour camera (Hamamatsu Photonics). Images were analysed with AquaCosmos software (Hamamatsu Photonics). For higher-speed observations, a custom-made system produced by the Dynamic-Bio Project was used. In this system, an Olympus IX-70 microscope was equipped with a special high-signal-to-noise-ratio colour confocal system (Yokogawa Electric), image intensifiers (Hamamatsu Photonics) and high-speed and ultra-high-sensitivity HARP cameras (NHK Engineering Service and Hitachi Kokusai Electric). In both settings, an Ar^+/Kr^+ laser (Melles Griot) was used to excite GFP at 488 nm and mRFP at 568 nm simultaneously. For 3D imaging, the objective lens was oscillated vertically to the sample plane by a piezo actuator system (Yokogawa Electric). Collected pictures were analysed with Volocity software (Improvision). Deconvolution analysis was also performed with Volocity by using theoretical point-spread functions optimized for CSU10.

To test the effect of a COPI mutation, GFP–Gos1 and Sec7–mRFP were expressed in wild-type and *ret1-1* cells. Cells grown to exponential phase at a permissive temperature (23 °C) were mounted on a glass slide and kept at a restrictive temperature (37 °C) on the microscope stage by the use of a thermo-control system (Tokai Hit Co.). Images were acquired 30–60 min after the temperature shift. Fluorescence intensities of the cisternae that were undergoing a green-to-red colour change during observation were quantified with AquaCosmos.

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Eleven cisternae from 8 wild-type cells and 14 cisternae from 7 *ret1-1* cells were subjected to kinetic analysis.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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