MICROSCOPY

Microscopy matures, and so does the Golgi

Two independent groups have applied fluorescence tagging and advanced video microscopy techniques to obtain visual evidence for the Golgi cisternal maturation model.

The Golgi apparatus—the organelle with the funny name—serves as a sort of post office for the cell, receiving newly synthesized proteins from the endoplasmic reticulum, appending post-translational modifications and then sending the modified proteins off to the correct cellular destination. For the last several decades there have been two models—the vesicular transport model and the cisternal maturation model—to describe how secretory proteins move from the early (*cis*) Golgi cisternal compartment to the late (*trans*) cisternae.

According to the vesicular transport model, Golgi cisternae are predicted to be stable, unchanging compartments, whereas they are thought to be transient, evolving structures according to the cisternal maturation model. Though biochemical evidence provided support for both theories (or in some cases, a hybrid of the two), visual confirmation by high-resolution live-cell imaging was missing.

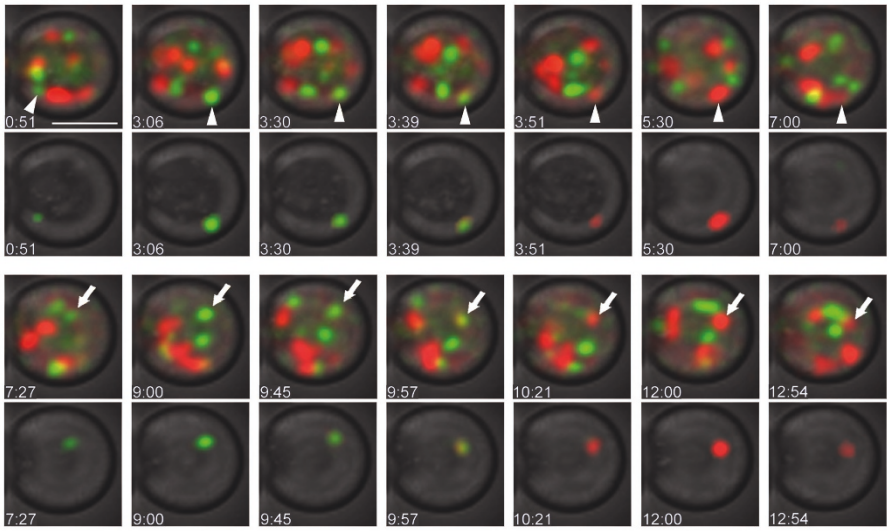

Figure 1 | Video microscopy frames showing that the resident Golgi protein composition of individual cisternae changes over time, in support of the maturation model (Losev *et al.*, 2006). Green, *cis* cisternae; red, *trans* cisternae. Scale bar, 2 μm. Reprinted with permission from Nature Publishing Group.

Now, as described in two papers published simultaneously in *Nature*, technical developments in confocal microscopy allowed the independent research groups of Benjamin Glick at the University of Chicago and Akihiko Nakano at RIKEN Discovery Research Institute in Japan to obtain live-cell images of fluorescently labeled Golgi cisternae (Losev *et al.*, 2006 and Matsuura-Tokita *et al.*, 2006).

Both Glick and Nakano chose to work with yeast cells, in which the Golgi cisternae are not stacked, allowing easier visualization of individual cisternae. The largest

PROTEOMICS

DISCOVERING NOISY PROTEINS

By using GFP-tagged yeast libraries in combination with flow cytometry, researchers can now obtain proteomic information at the single-cell level, revealing the underlying structure of biological noise.

Although DNA microarrays have helped to revolutionize genomic analyses, they are far from being able to provide a complete picture of the inner workings of a cell. For example, it is well known that mRNA levels do not always correlate with expressed protein levels. Proteomics techniques yield much richer information about cellular behavior than measurements of mRNA levels alone, and are welcome additions to the cell biologist's toolbox.

"If you want to have any understanding about how a cell works, you have to understand where noise comes from, how prevalent it is, and what its biological effects are," says Jonathan Weissman of University of California at San Francisco who, along with postdoc John Newman, devised a flow cytometry–based method to investigate the variation of protein expression on a 'global' scale. Working with a previously established GFP-tagged yeast library, in which each protein is expressed as a C-terminal GFP fusion from its endogenous promoter at its natural chromosomal position, the researchers developed custom software to control the delivery of cells to a flow cytometer, make quantitative fluorescence measurements and analyze data.

Their system allowed detection of about 60% of the yeast proteome, or more than 2,500 proteins, a value that Weissman believes can be improved upon by using a red fluorescent protein variant (to evade the problem of autofluorescence). The measured fluorescence of each cell accurately reported the abundance of the tagged protein; cell-to-cell variation, or 'noise', in protein expression could be readily calculated from the flow-cytometry data. "Not only were we able to get very high-quality proteomic information, but we were able to get it with single-cell resolution,

technical hurdle was to develop advanced imaging techniques; both groups developed custom-built three-dimensional confocal video microscopy systems allowing multicolor live-cell imaging at unprecedented resolution. "We can now clearly observe the tubular network structure of yeast Golgi cisternae, which we never imagined to see without the use of electron microscopy," says Nakano. Both groups used GFP to label a *cis* Golgi resident protein and a red fluorescent protein variant to label a *trans* Golgi protein (or vice versa). They hypothesized that if proteins moving through the Golgi are transported by vesicles, then the green fluorescence marking an individual *cis* cisterna should be retained indefinitely. If cisternae mature from a *cis* state to a *trans* state, however, then the fluorescence should change from green to red as cisternae acquire new biochemical properties.

Both groups clearly observed that individual green *cis* cisternae changed color to red over a matter of minutes (**Fig. 1**). "The color change clearly ruled out the classic simple vesicular transport model," explains Nakano. Glick was also surprised that the visual evidence for cisternal maturation was so clear-cut. "We had been concerned that Golgi cisternae might undergo frequent fusion and fission, and that these events would complicate the interpretation," he says.

The success Glick and Nakano have had in answering a long-standing question in cell biology is also an excellent example of how working together rather than racing to compete for the same goal can provide fruitful results. "These papers will be scrutinized closely, so it was important to take the time to do the experiments right," says Glick. "The fact that our two groups independently reached similar conclusions is reassuring and should also make the story more persuasive to others in the field."

Allison Doerr

RESEARCH PAPERS

Losev, E. *et al.* Golgi maturation visualized in living yeast. *Nature*; published online 14 May 2006.

Matsuura-Tokita, K. *et al.* Live imaging of yeast Golgi cisternal maturation. *Nature*; published online 14 May 2006.

which is something that DNA microarray experiments are not capable of doing," explains Weissman.

This clever experimental design allowed Weissman, Newman and their colleagues to make global observations about the structure of biological noise in the cell. For example, proteins exhibiting very low noise included those involved in translation and protein degradation. Proteins with noisy expression included those involved in chromatin remodeling. Not surprisingly, some of the proteins with the most variable or noisiest expression were the 'first responders' to changing environmental conditions, such as those related to stress response and heat shock. Weissman remarks, "The implication is that this may be a way for cells to generate diversity in the phenotype even under one condition, so that if conditions change, there will be at least a subset of cells that will be better optimized to respond to that change."

Allison Doerr

RESEARCH PAPERS

Newman, J. R. S. *et al.* Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise. *Nature*; published online 14 May 2006.

NEWS IN BRIEF

MICROARRAYS

A robust small-molecule microarray platform for screening cell lysates

Bradner *et al.* present a process for the simplified construction of covalently linked small-molecule microarrays that present a diverse variety of natural and synthetic compounds. They subsequently show how the screening process can be simplified by using whole lysates from cells in which the protein of interest is expressed with an epitope tag or as a fluorescent chimera.

Bradner, J.E. *et al. Chem. Biol.* **13**, 495–504 (2006).

BIOINFORMATICS

Computational redesign of endonuclease DNA binding and cleavage specificity

Working with a computational strategy that allows atomic-level modeling of protein-DNA interfaces, Ashworth *et al.* designed a variant of the homing endonuclease I–*Mso*I with altered target preference. This modified enzyme displayed 10,000-fold enhanced binding and cleavage of an alternate site, with target-discrimination capabilities that are equivalent to the wild-type enzyme.

Ashworth, J. *et al. Nature* **441**, 656–659 (2006).

PROTEOMICS

A pharmacological map of the PI3-K family defines a role for p110 in insulin signaling

The 15 kinases of the phosphoinositide 3–kinase (PI3-K) family are recognized as important drug targets, but the physiological roles of individual kinase isoforms are poorly understood. Knight *et al.* screened a large library of PI3-K inhibitors, revealing details about target specificity, which could provide valuable insights into isoform-specific function and assist in future drug-design efforts.

Knight, Z.A. *et al. Cell* **125**, 1–15 (2006).

IMAGING AND VISUALIZATION

Virtual histology of transgenic mouse embryos for high-throughput phenotyping

Magnetic resonance microscopy (MRM) is a powerful tool for embryological imaging, but it may not be a practical option for large-scale screening. Johnson *et al.* present a less expensive alternative for high-throughput work, using X-ray microscopic computed tomography (microCT) with osmium tetroxide–stained mid-gestation embryos to achieve rapid, high-resolution imaging.

Johnson, J.T. *et al. PLoS Genet.* **2**, e61 (2006).

GENOMICS

Regulatory blueprint for a chordate embryo

Previous *in situ* hybridization studies have identified 76 genes regulating embryogenesis in the tunicate *Ciona intestinalis*. By analyzing gene-expression changes in response to disruption of these genes, Imai *et al.* assembled a detailed map of the genetic circuits underlying the formation of various organs and tissues, information that could yield important insights into chordate evolution.

Imai, K.S. *et al. Science* **312**, 1183–1187 (2006).