OBSERVING TRAFFIC WITHIN CELLS

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Ever since membranes could be resolved within cells, the pattern of intracellular membrane trafficking has been under investigation. Images made with the transmission electron microscope revealed numerous small vesicles that appeared to be shuttling between the endoplasmic reticulum (ER) and the Golgi complex. However, these static images offered no information as to the direction the vesicles were moving and were quite inconclusive. Nevertheless, evidence built over time to suggest that unrefined proteins were moved in small vesicles from one intracellular compartment to another. Recently, a study by John Presley,[1] Nelson Cole, Trina Schroer, Koret Hirschberg, Kristen Zaal, and Jennifer Lippincott-Schwartz[2] with the light microscope has challenged this view. They not only presented convincing morphologic data in their published article, but they also posted some Quicktime movies on a Web site (http://dir.nichd.nih.gov/CBMB/pb1labob.html) that are even more convincing.

In a variety of cultured cells (COS, HeLa, NRK, MDCK, CHO, and primary glial cells), Presley *et al.* transiently expressed a tagged protein. Specifically, this was VSVG protein that is known to undergo predictable conformational changes at certain temperatures. By controlling the temperature, thereby controlling the conformation of the VSVG protein, they could retain the protein in the ER or allow it to be moved to the Golgi. The VSVG protein was tagged with green fluorescent protein (VSVG-GFP) and experiments showed that the fluorescent label did not effect the intracellular movement of the protein. Movement of tagged proteins was followed with a confocal microscope system or a microscope equipped with a cooled charged-coupled device. Fluorescent material could clearly be seen moving from the ER to the Golgi.

Using temperature shifts to "turn on" the ER-Golgi transport, Presley *et al.* followed the complete life history of individual transport intermediates in living cells, including their formation, path, and velocity *en route* to the Golgi. The shape of the transport intermediates varied from tubular to vesicular, with some of the tubules being rather contorted. In the Quicktime movies (filmed in time lapse, *e.g.*, fluorescent images captured at 3.6 second intervals) dynamic images showed particles of various shapes flowing from all over the cell (except the nucleus) to gather at the Golgi. It was reminiscent of a cosmic implosion. It could be appreciated that a tubule could appear to be a vesicle in a thin section examined with the electron microscope.

Some cells were treated with nocodazole, a drug that depolymerizes microtubules. The fluorescent material simply quivered in place (Brownian motion?) rather than streaming toward the Golgi. This strongly suggested that the ER to Golgi traffic passed along a scaffold of microtubules. In additional experiments, the molecular motor driving the traffic was investigated. Cytoplasmic dyneins power movement toward the minus end of microtubules, which was the direction toward the Golgi in these experiments. Overexpression of another molecule that disrupted the dyneins prevented the fluorescent material from aggregating at the Golgi. These and other experiments led to the conclusion that packages of different sizes and shapes (tubules or vesicles) moved along microtubules, driven by dyneins, from the ER to the Golgi.

In addition to providing important biologic information, this study nicely demonstrates the power of the Web for disseminating dynamic information. The Quicktime movies were fascinating to watch. If a static image is worth 10^3 words, then perhaps a dynamic image is worth 10^6 words!

1. The author gratefully acknowledges Jeff Salisbury for helpful discussions (including the concept of studying science *in silico*) and Jennifer Lippincott-Schwartz for reviewing this article.

2. Presley, J.F., N.B. Cole, T.A. Schroer, K. Hirschberg, K.J.M. Zaal, and J. Lippincott-Schwartz, ER-to-Golgi transport visualized in living cells, Nature 389: 81-85,1997.

Front Page Image

APHIDS FROLICKING ON BLUE GREEN ALGA (*SCYTONEMA*)

by Tina (Weatherby) Carvalho, Biological EM Facility, University of Hawaii

The original aphid came from my herb garden, the alga from a culture in the natural products chemistry lab here at the University of Hawaii. The two organisms never really met, and there is actually a huge difference in magnification between them. So how did they get together in this picture?

The original scanning electron micrographs were, of course, in black and white, and each of the aphids were cloned from a single picture. This image was my first effort at making a composite with Adobe Photoshop, and is primitive in that there are no drop shadows or other manipulations that would help to convince you that the image was real. But make no mistake; with today's ubiquitous image editing software, I could concoct something very convincing; I could have replaced OJ's shoes with Dorothy's red slippers, and make you believe it!

For further information on this process, refer to Ms. Carvalho's article on page 10 of this issue.

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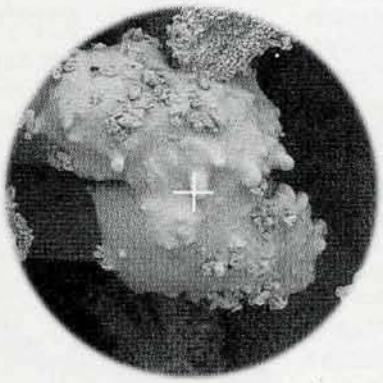

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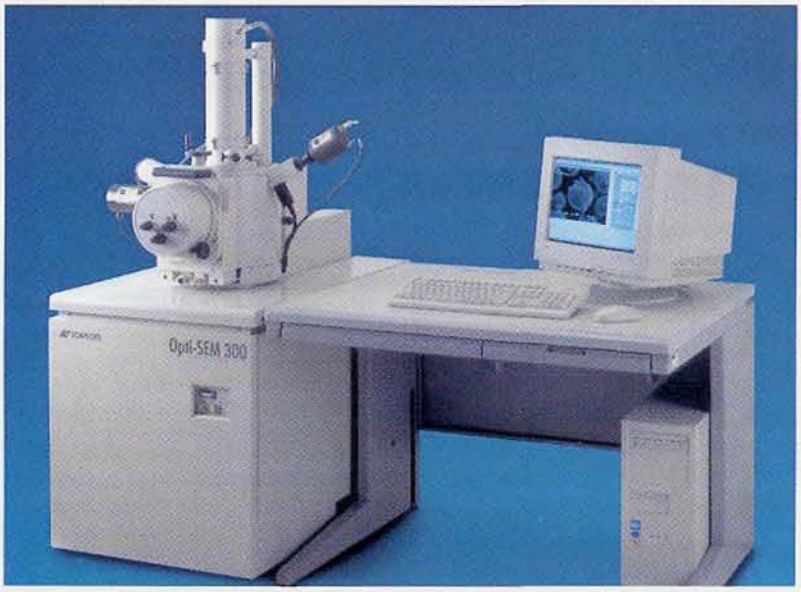

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