MOVING VESICLES FROM THE GOLGI

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We know that material within a cell is "packaged" within the Golgi apparatus. These "packages" (vesicles) bud off from the trans face of the Golgi and proceed to their destination. Just what drives this budding off process is closer to being understood, thanks to a recent report by Steven Jones, Kathryn Howell, John Henley, Hong Cao, and Mark McNiven.[2] They presented morphologic and biochemical evidence that dynamin plays a role in this process.

The dynamins are a family of 100-kD guanosine triphosphatases that are thought to be involved in the budding off of vesicles from the plasma membrane during endocytosis. Whereas some forms of dynamin are restricted to specific cells, such as neurons, the form known as dynamin II (Dyn2) is present in all tissues, including epithelium. Jones *et al.* linked Dyn2 to green fluorescent protein (GFP) and expressed it in cultured epithelial cells from the liver. Living and fixed cells were examined with an epifluorescence microscope or a confocal laser scanning microscope. In parallel, cells in which the Dyn2-GFP had not been expressed were examined by immunohistochemical techniques for a conserved region of dynamin. In both studies, a prominent punctate staining was seen at the plasma membrane, which was expected from earlier studies, and in the Golgi region, suggesting that Dyn2 is localized to vesicles at both the plasma membrane and the Golgi complex.

In a follow-up study, cells expressing the Dyn2-GFP were labeled with antibodies to either clathrin or TGN38, an antigen associated with the trans face of the Golgi. Clathrin, which is known to decorate the outside of vesicles budding from the plasma membrane and nascent vesicles budding from the Golgi, was shown to have essentially the same distribution as the Dyn2-GFP. The Dyn2-GFP and TGN38 appeared to be co-localized in the central portion of the cell near the Golgi, but not peripherally near the plasma membrane.

Complementary biochemical studies were done to support these morphologic findings. A highly enriched rat liver Golgi fraction was used to measure the binding of dynamin under three conditions, but cytosol was present in all: in the absence of adenosine triphosphate (ATP), with added ATP, and with an ATP regeneration system either with or without guanosine 5'-O-(2-thiodiphosphate)(GTP-γ-S). When dynamin was incubated with just the cytosol, very little binding was found associated with the Golgi fraction. Adding the ATP or the ATP regenerating system caused a three-fold increase in dynamin binding, and the addition of GTP-γ-S increased the binding ten-fold. This supported the morphologic evidence that dynamin is associated with the Golgi and further demonstrated that this association is energy-dependent.

Another biochemical study showed that antibodies to dynamin inhibited the formation of vesicles in a cell-free assay rich in trans-Golgi network, and control antibodies did not. In additional studies, the assay was performed with cytosol immuno-depleted of dynamin and the formation of exocytotic and clathrin-coated vesicles was totally inhibited. Re-addition of dynamin restored the budding activity of both types of vesicles in a dose-dependent fashion. Adding dynamin without the depleted cytosol did not restore budding activity, indicating that there are other factors in the cytosol, in addition to dynamin, that are required for budding.

These morphologic and biochemical studies clearly suggest that dynamin play a role in moving vesicles from the Golgi complex. Although this role is not yet precisely defined, Jones *et al.* speculated that dynamin may snip the vesicles from the trans face of the Golgi.

1 The author gratefully acknowledges Dr. Mark McNiven for reviewing this article.

2 Jones, S.M., K.E. Howell, J.R. Henley, H. Cao, and M.A. McNiven, Role of dynamin in the formation of transport vesicles from the trans-Golgi network, *Science* **279**:573-577, 1998.

Front Page Image

Spider Web Silk Spinnerets

This micrograph was taken by a group of fourth grade students from Punahou School in Honolulu in May of 1995. They were participating in the school's Spring Fling, an event where parents may volunteer to give a week-long class in any subject, craft, or sport. This workshop, entitled "Snoop with a Loupe", given by Tina Carvalho at the Biological EM Facility at the University of Hawaii, was based on ideas from The Private Eye Project. This program, developed by Kerry Reuf and David Melody, is designed to foster creative thinking in science, art, and writing. Students use a jeweler's loupe to explore the things around them and then to ask "What else does it remind me of? What else does it look like?" They were asked to make a list of analogies, which could then become Bones-for-a-Poem or stimulate ideas for a story or drawing. Creativity in writers, poets, scientists and artists often comes from a heightened awareness of the things around them, and we hoped that by introducing our eight Spring Fling students to a change of scale and a new perspective we could lead them to find some of the wonder and drama of the world. The week culminated in a visit to the EM Facility where they looked at some of their favorite specimens in the SEM and at forming crystals in the light microscope (but mostly they wanted to play with the liquid nitrogen!).

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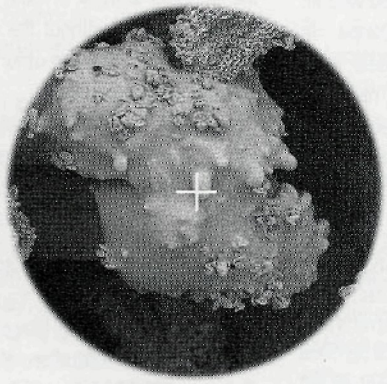

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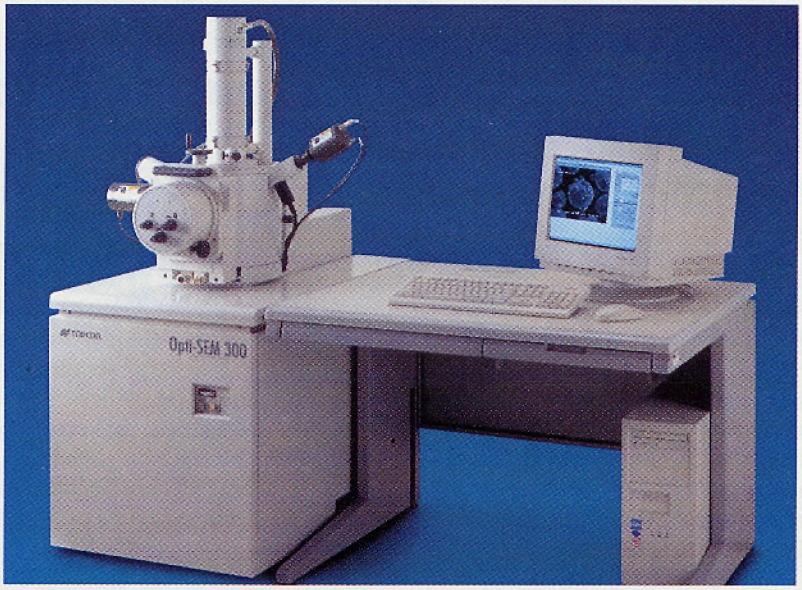

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