Live Cell Imaging of Endoplasmic Reticulum and Golgi Dynamics in Higher Plants

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In plant cells the Golgi apparatus is in the form of numerous individual stacks of cisternae scattered throughout the cytoplasm. A combination of live cell imaging and expression of fluorescent protein constructs, both transiently and stable [1], has shown that Golgi bodies are normally associated with tubules of the cortical endoplasmic reticulum [2] which overlie an actin network. Surprisingly Golgi bodies are motile over the ER network which in itself shows an unexpected level of motility.

We have shown that a number of arabidopsis class XI myosins are involved in Golgi movement [3]. Expression of dominant negative forms of the myosin lacking the actin binding domain at the amino terminus perturb organelle motility, but do not completely inhibit movement. Application of latrunculin B, an actin destabilising drug, inhibits Golgi movement to a greater extent compared with the affects of arabidopsis myosin XIE/XIK expression. XIE and XIK constructs are dispersed throughout the cytosol and do not completely decorate the Golgi. Also, they do not affect the global actin architecture, but their movement and location is actin dependent.

Although Golgi bodies are motile over the ER, the proteins composing the ER exit site also stay attached to the Golgi and form a motile secretory unit. Photobleaching has demonstrated that cargo transport from ER to Golgi can take place in both static and motile Golgi stacks [4.]. Photoactivation of GFP has also been used to determine that proteins within the membrane of the ER flow as the ER is remodeled [5]. The rate at which activated GFP moves away from the activation spot has shown that this motion is much faster than would be expected if membrane proteins moved simply by diffusion. This implicates myosin in the movement of not only the ER tubules that continually remodel but ER membrane proteins themselves. Tracking of Golgi body movement demonstrated that they move at the same rate and in the same direction as do photoactivated ER surface proteins. Golgi bodies, therefore, probably move with, and not over, the surface of the ER. Using the same truncated myosin constructs that were applied to study Golgi movement we have also shown that ER movement and remodeling is indeed myosin dependent.

By the application of infrared laser optical traps to individual Golgi bodies within living arabidopsis leaf cells, we have shown that Golgi bodies can be micromanipulated to reveal their association with the ER [6]. Golgi bodies appear to be physically attached to ER tubules and displacement of individual Golgi bodies by moving the laser trap results in the rapid growth of any attached ER tubule (see figure 1). Thus the ER network can be remodeled in living cells simply by movement of laser trapped Golgi dragging new ER tubules through the cytoplasm and new ER anchor sites can be established. Trapped Golgi ripped off the ER are “sticky” and can be docked on to and attached to ER tubules, suggesting the presence of tethering factors on the Golgi that aid in attachment to the ER.

In a separate project we have been investigating the distribution and potential tethering role of a number of plant coiled-coil Golgi matrix proteins (Golgins) some of which may be responsible in mediating the close Golgi/ER interaction. Using fluorescence

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resonance energy transfer combined with fluorescence lifetime imaging we have shown interactions of various matrix proteins with small GTPases (Rab and Arl1) which are implicated in the regulation of binding Golgins to Golgi membranes [7].

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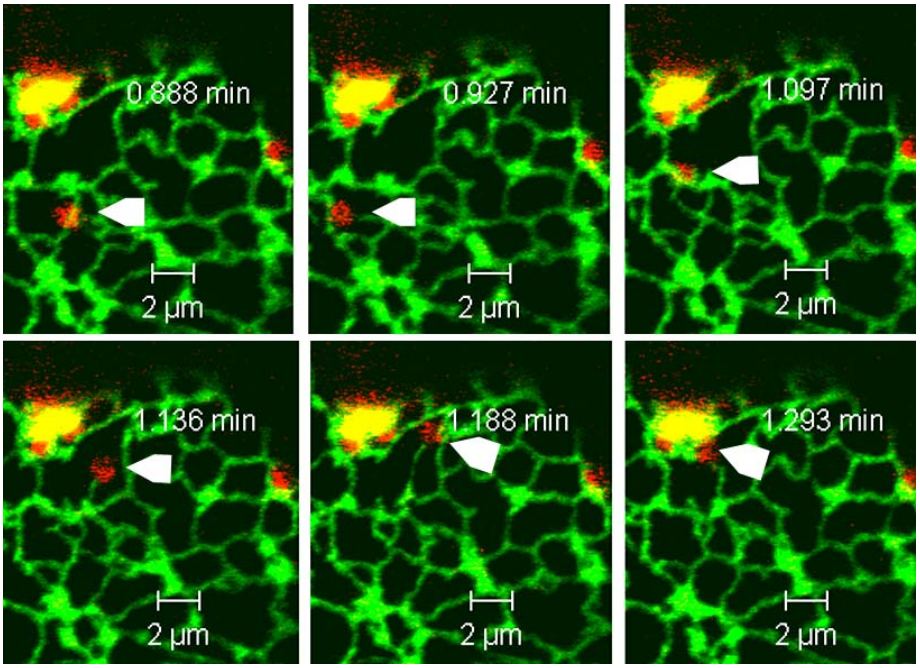

Figure 1. Laser trapping of Golgi bodies in an arabidopsis leaf epidermal cell. The plant was transformed with a GFP-HDEL (ER labeling) and ST-mRFP (Golgi labeling) constructs. A Golgi body is trapped by the laser beam (arrow head) and moved through the cortical cytoplasm dragging a new ER tubule behind it. Thus the Golgi bodies can be firmly attached to the ER.

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