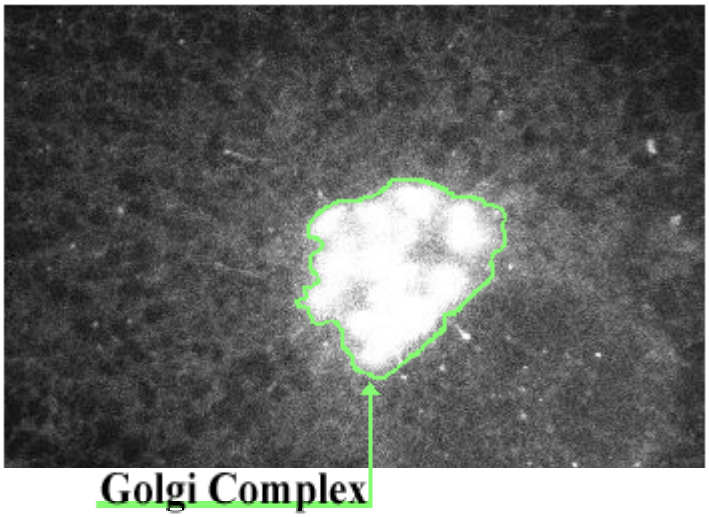
Video Problem

4. The movement of newly synthesized membrane protein through the complex array of intracellular membranes presents interesting questions of both sorting and motility. Recently, it has been possible to tag newly synthesized protein "naturally" with a fluorescent tag by inserting the nucleotide sequence for Green Fluorescent Protein (GFP, a jelly fish protein) to one end of the gene coding for the protein of interest. Following translation, GFP spontaneously folds into a fluorescent "tag" that in many instances does not inhibit the subsequent processing or ultimate structure or function of its chimeric partner.

The figures and videos that follow were produced from time-lapse observations of the progression of newly synthesized, GFP-labeled vesicular stomatitis virus membrane protein(GFP-VSVP) from ER to the Golgi complex in cultured COS cells. Very thin optical sections of each cell was obtained by confocal microscopy. In these images, a faintly fluorescing ER network surrounds a collection of much brighter, large vesicles that constitutes the Golgi complex. In all instances the Golgi complex, when present, is situated adjacent to the nucleus. Images in the first video were captured by a digital camera about every 9 sec; they are displayed in the quick-time movie at a rate of about 9 frames a second and thus are speeded up about 81-fold. The second video lasts 18 sec, and runs at about 12 frames per sec; its images were captured about every 4 sec, and the movie therefore speeds up the natural process about 48-fold.

Recall what you know about the steps of post-translational processing, click on the still image below to bring up the movie, watch the movement of GFP-VSVP into the Golgi several times (at least once, one frame at a time) and answer the questions in the right-hand frame.



[hyperlink to //Presley1ERGolgi.mov]

Questions

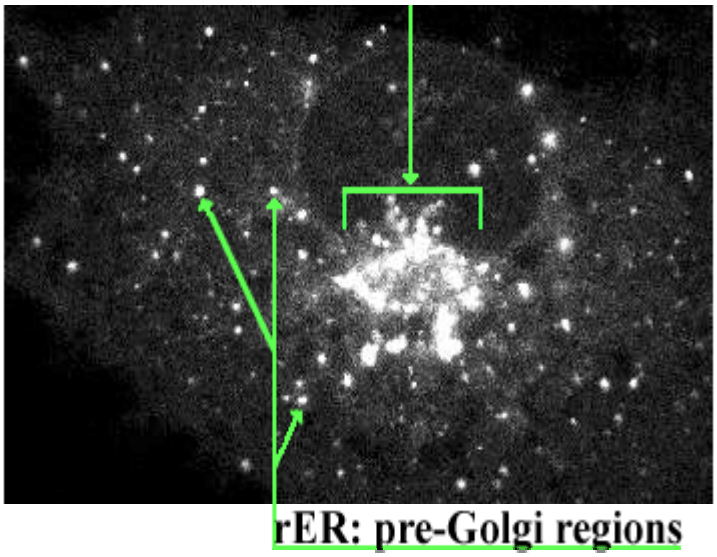
A. Describe carefully the movement of GFP-VSVP with respect to the Golgi complex.

B. Critically compare your observations here with your expectations based on text and lecture.

C. To obtain more information about this process, cells were cooled for several hours, which retarded translocation more than translation. Consequently, newly translated GFP-VSVP accumulated in pre-Golgi regions prior to heating. Turn the page [hyperlink to next set of frames] to view the results of this experiment.

You can learn more about these phenomena and the following experiments by consulting the video essay by Presley, *et al.* 1998 **Mol Biol. Cell** 9:1617-1626. PMID9658158

4.1. Temperature-shift Experiment:



[hyperlink to PresleyPreGolgi.mov]

Questions

A. Once again, describe the movement of the tagged protein as the temperature is raised.

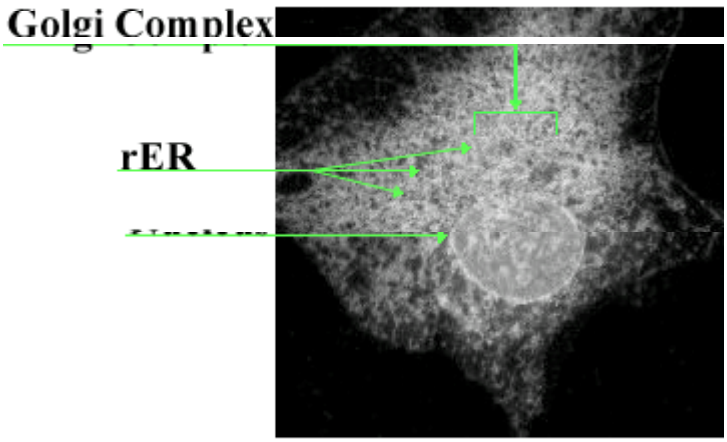
B. How do your observations compare with those made from watching the first video?

C. How might you determine whether GFP-VSVP is diffusing randomly from ER to Golgi?

D. If you think the movement is non-random, how might it directed. Turn the page [hyperlink to new page with following three frames] to consider the results of pretreating the COS cells with nocodazole.

4.2. Nocodazole Pretreatment:

To investigate what causes the ER-to-Golgi movement of GFP-VSVP, COS cells were incubated for 15 min in iced culture medium containing nocodazole (1 µg/ml), washed free of nocodazole and incubated at 32 oC in regular growth medium. For comparison purposes, the previous figure and video are presented in the left-hand frame, the nocodazole figure and video in the center frame. Consider both videos, noting the great difference in time elapsed, and answer the questions posed in the right-hand frame. The video images were captured every 4 min. The movie lasts about 9 sec, is run at 4 frames per sec, and the movement of the fluorescent protein is greatly speeded up about 240-fold.



[hyperlink to Presley3Nocodazole.mov]

Questions

1. Describe the movement of GFP-VSVP following nocodazole treatment and compare it with that seen in the previous video (which can serve as a Control).

2. Given these results how is transport from ER to Golgi likely caused?

3. How would you determine what motor mechanism is involved?