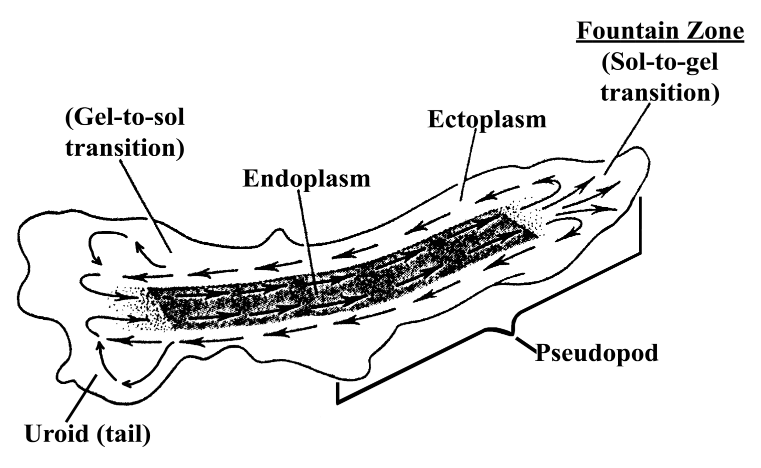
Cytoskeleton and Cell Motility

1. (28 pts) *Amoeba proteus*, a single-celled eukaryote, moves by means of psudopods attaching to and detaching from the substratum. Locomotion seems to be correlated with the forward flow of fluid cytoplasm (endoplasm) into an advancing pseudopod through a surrounding, gel-like ectoplasmic tube. The ectoplasm forms at the pseudopodial tip in a region called the Fountain Zone. As the amoeba advances the ectoplasmic tube “liquifies” at the posterior end to form endoplasm. These features are illustrated in the figure below.



Both locomotion and cytoplasmic streaming are inhibited by cytochalasin B.

Consider everything you’ve learned so far and answer all the following questions.

A. (4 pts) When amoeba undergoes cell division, it stops streaming and rounds up into a spherical cell. Describe how this change in shape and behavior comes about and why it might be a necessary precondition for division.

B. (6 pts) Briefly describe how cytoplasmic streaming is most likely organized and generated at the cellular and molecular levels.

C. (5 pts) Briefly describe an additional experiment or observation that would test your hypothesis and indicate clearly what the results would show.

D. (8 pts) Describe clearly, with the aid of a well-labeled diagram, how streaming *within* a pseudopod could result in movement of the amoeba across the substratum.

E. (5 pts) Describe how your streaming mechanism might be regulated such that the amoeba might change its streaming pattern to form phagocytic pseudopods around a ciliate it had touched.

Now evaluate some past answers to these questions, in light of your own essays. Note that better answers contain more information that you have covered at this point in the course. On the other hand, you may now know more about the various mechanisms than the students who answered these questions in the mid ‘90’s did!

A. (4 pts) When an amoeba undergoes cell division, it first stops streaming and rounds up into a spherical cell. Describe how this change in shape and behavior comes about and why it might be a necessary precondition for division.

Answer	**Comment**
Example 1. In order for the single cell to divide it must become a shape that is spherical enough for the spindle to form and an even distribution of cytoplasmic material to take place when cytokinesis happens. Also, cytokinesis cannot take place with a firm gel-like tube in the middle of the cell. In order for cell division to occur, the gel-like tube will dissolve into all endoplasm which is a liquid form. The cytoskeleton will form as a normal eukaryotic cell and cytokinesis will divide the cell. Once the cell has full divided, the endoplasm will form a new ectoplasm tube again and all will continue	**A good start, but more mechanistic detail is required: what sorts of cytoskeletal elements are involved?** **Wordy! Simply restates information provided.** **“normal” is vague – what does it mean in this context?**

Contrast the first answer with the following:

Example 2. Microfilaments are needed for cytokinesis. They form a “belt” perpendicular to the spindle fibers needed for mitosis. This belt contracts, pinching off the cytoplasm from the original cell into 2 daughter cells. Microfilaments are dynamic structures, and those which previously were involved in streaming or maintaining cell shape are disassembled and used for cell division. When this occurs, the cell assumes a natural round shape and all streaming stops due to lack of microfilaments which would turn the endoplasm into ectoplasm.	**A well-focused mechanistic answer from the start!** **Connection with streaming established; dynamic properties identified.** **A problem: what is “natural?”**

How did your answer to the Question B. compare with those on the next page?

B. Briefly describe how cytoplasmic streaming is most likely generated and organized at the molecular and organelle level.

Example 1. The endoplam is a basic component of ectoplasm. This, the association of many endoplasmic forms ectoplasm in a similar way [that?] F-actin makes up G-actin to form microfilaments. The assocation forms a gel-like tube that is unstable at both ends. When endoplasm is in its component form, it is a liquid. However, when it associates with other endoplasms to make ectoplasm it forms a gel. The movement of the organism is caused by the breakdown or dissociation of the ectoplasm gel tube into its liquid endoplasm at the posterior end of the tubule causing fro?? pseudopod. This endoplasm then flows through the remain gel ectoplasm tube. The breakdown always happens at the posterior or (-) end. For each molecule of ectoplasm that dissociates, another molecules of endoplasm will associate at the (+) end toward the pseudopod. This allows the ectoplasmic tube to remain about the same length while the endoplasm at the end of the tube is allowed to flow all the way to the front. The plasma membrane is fluid so it conforms to the changing shape of the org[anism?].	**Garbled fact: G-actin is a subunit of F-actin (microfilament).** **This is an interesting but unfocused essay. It is interesting insofar as it attempts to relate various aspects of streaming to cellular locomotion, BUT this discussion is irrelevant to the question asked. What the likely "motors" are and where are they located are not addressed.** **Confusing polarity of MT and MF organelles with cellular polarity.** **The handwriting was actually difficult to decipher, and grammatical errors increased the reader's difficulties.**

By comparison, what do you think of this answer? Add your comments in the space provided.

Example 2. Pseudopod extentions can be generated throug the interaction of actin (MF) and myosin. High ATP and Ca levels could be present at the pseudopod which would allow myosin to be phosphorylated. The actin would be forming microfilaments from G-actin because of the high ATP concentration at the pseudopod. Once phosphorylated, myosin could interact with the actin microfilaments to produce the force necessary to extend the pseudopod. As the pseudopod extends fluid endoplasm would move into the pseudop. The presence of a high conc. of Ca/calmodulin at	

the sol-to-gel transition could cause myosin to be phosphorylated by its light-chain kinase. A low conc of at the gel-to-sol transition area could cause the gel cytoplasm to beome fluid, allowing it to flow toward the pseudpod. It could be come fluid as actin at the (-) end of the MF depolymerizes or as myosin filaments disassociate do to dephosphorylation. Once disassociated, the subunits (G-actin and free myosin) could move in the fluid cytoplasm to where it is needed again for further streaming.	

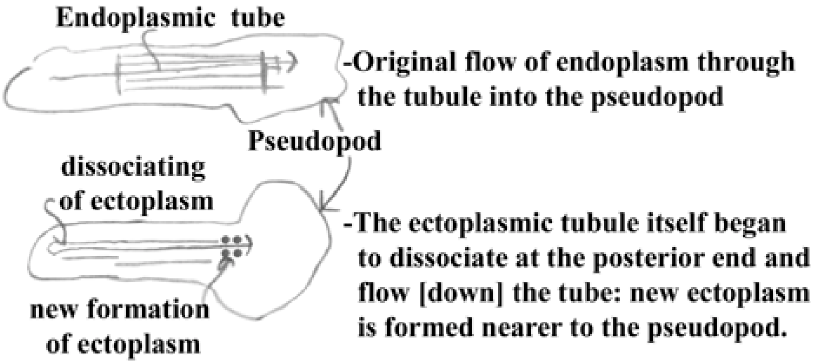
The validity of these two mechanisms were tested, respectively, in the following two examples of answers to **part C**. What do you think of them, as applicable to the two answers and more generally?

Example 1. To test this hypothesis, an inhibitor could be added to inhibit the breakdown of “liq??ficati??” of the ectoplasmic tube. We know that the breakdwon of the ectoplasm can be stopped or inhibited by cytochalasin B. When cytochalasin B is added to the cell I [verb???] gradually the movement of the cell and cytoplasmic streaming will stop (and it does as stated above) because no more breakdown of the ectoplasm is possible. Also we may want to mark one molecule with a radioactive pulse label. By doing this, one could follow the passage of the endoplasm from the (+) end of the ectoplasm all the way to the (-) end and the dissociation of the endoplasm and as travelling down the ectoplasm tube to the pseudopod and its gradual incorporation back into the ectoplasm. This is an even more visible experiment, that should show a similar effect as the treadmilling of microfilaments.	
Example 2. A good way to test this hypothesis would be to eliminate the transformation from ectoplasm to endoplasm, for the contraction this results in is what powers the streaing., The polkymerization of globular actin into filamentous actin depends	

on the ratio of G-actin-ATP/G-actin-ADP subunits. If much G-actin-ADP was introduced into the system, say by microinjection, right after the formation of a new *A. proteus*, the concentration of G-actin-ATP would be so low that polymerization into microfilaments should be greatly inhibited. If this is the case, endoplasm-ectoplasm transition will never take place and the basis for cytoplasmic streaming can never be established. This will show that streaming is caused by the contraction resulting at the sol-gel transition. Introducing cytochalasin B, which interferes with microfilament assembly, could test this hypothesis as well, but I assumed you wanted to hears something else since cytochalasin B was mentioned in the question.	

Question D. asks you to relate streaming *within* a pseudopod to locomotion of the whole cell across the substratum. Critique the two answers that follow and note in particular whether the qualities of the essays and diagrams are at all correlated. **Note** also an important feature of locomotion is missing from all 3 answers, which is important for answering Question E.

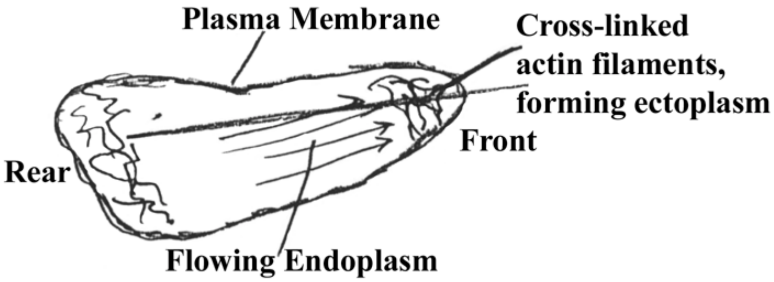
Example 1. (*Note hand-written labels have been replaced with printed ones.)*



	Comments?
There is a continuous breakdown of the ecoplasm and regeneration at the end closest to the pseudopod. This causes the pseudopod to keep moving in the direction of the assembly/disassembly of the ectoplasm.	

Example 2.

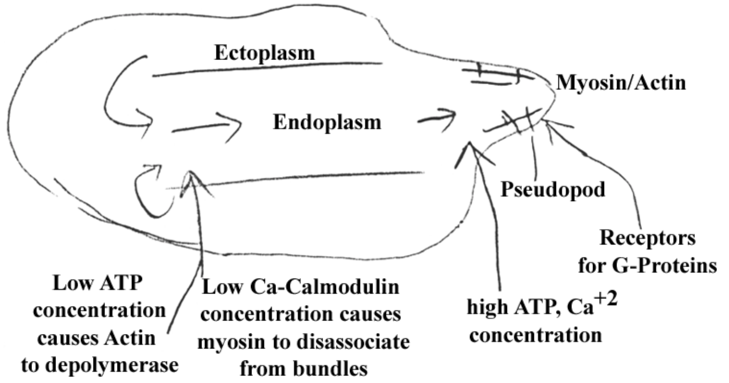
Comments



As the endoplasm is drawn toward the pseudopod to fill the space left by the contracting ectoplasm, it could exert pressure on the end of the pseudopod, pushing it forward. In addition, there could be a new movement of cytoplasm from the "rear" of the organism to the "front". This would result in the pseudopod extending and bringing the rest of the amoeba along with it. This would result in the movement of the organism across the substratus.

Example 3.

Comments



As the pseudopod extends through myosin/actin interactions the cytoplasm would be converted to fluid because of the low Ca-calm complex concentration.
Because of the net flow of cytoplasm in one direction the amoeba would tend to move across the substratum.

Question E. concerns the *regulation* of streaming, using a change in locomotory behavior for feeding as an example. The best answers derive details from examples and focus on the general features of the problem.

	Comment
Example 1. It would be regulated by the direction and speed of the movement of the pseudopod. If the movement of the organism were quick, the breakdown or assembly of the ectoplasm would obviously be quick. However, the organism can only change direction be moving the pseudopod to the other end of the [???] through the channel and switching the breakdown/assembly of the ectoplasm polarity. The creature moves around the ciliate by engulfing it by phagocytosis. This occurs by the ectoplasm breaking down until has completely surrounded the ciliate and then reactivating once the cell has ch[????] it	

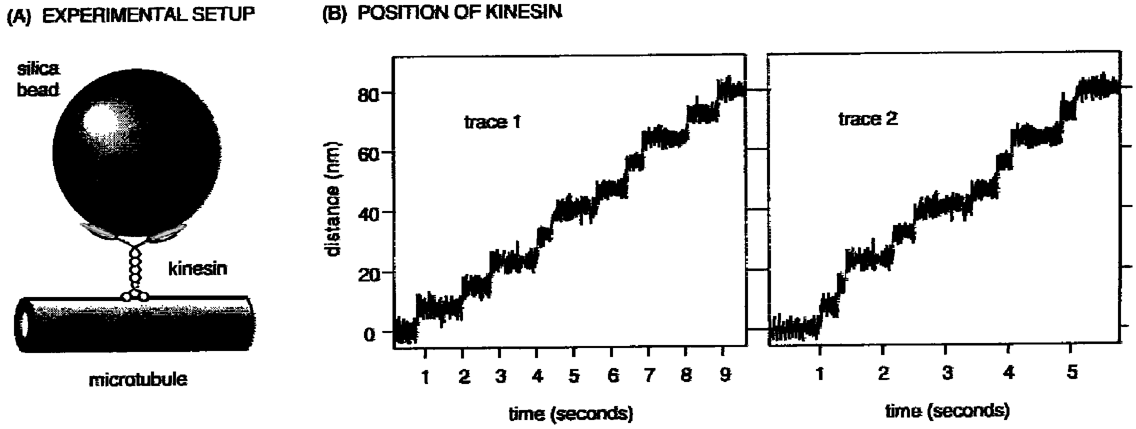
Example 2. There could be receptors on the plasma membrane which would detect when it touched a ciliate. When this occurred, the direction of the streaming could be changed to move towards the ciliate. The pseudopod would move toward the ciliate, its plasma membrane enveloping it as it got closer. When the membrane completely surrounded the ciliate, the inner and outer membranes created could fuse with themselves, creating a closed pseudopod with a phagocytotic vacuole inside that could be brought to a lysosome for digestion. The streaming toward the ciliate could be regulated by controlling ATP-G-actin concentrations, making them higher therefore creating more actin filaments, increasing sol-gel transformations and the pressure that creates pseudopod extension. Perhaps the receptors activate a second messenger which in turn activates the polymerization of F-actin.	

2. (28 pts) While you're enjoying the “intense” Spring sunshine in Vermont, I thought you might like thinking about some interesting data generated by the use of polarized laser energy, another very intense form of illumination. I've modified the problem from one that appears in **The Problem Book** (which accompanies **Molecular Biology of the Cell** by Alberts, *et al*).

The movement of single motor proteins can be monitored using polarized laser light. Such illumination creates a circular interference pattern with a circular force field that ranges from zero at the center to a few piconewtons at the periphery (about 200 nm from the center). Individual molecules entering this interference pattern are pushed towards the center and "captured" there, unless they possess sufficient kinetic energy to escape. These interference patterns are often called "optical tweezers" because scientists can use them to move molecules about, simply by repositioning the interference patterns. And the actual work accomplished by motility mechanisms can be estimated from the force necessary to escape such constraints. Nifty!!

One experiment used optical tweezers to position a kinesin molecule on a microtuble attached to a coverslip (as illustrated below in Fig. A.). The behavior of the kinesin could not be resolved microscopically but its movement was readily tracked by attaching a much larger silica bead to it. When incubated in an appropriate physiological solution, the bead vibrated with the kinesin molecule, due to thermal kinetics, but it did not move away from the center of the interference pattern. When ATP was added to this test set-up, the bead began to migrate away from the center and towards the one end of the microtubule.

As it moved along the microtubule, kinesin encounterd the force of the interference pattern, which is thought to simulate the kind of loads born by kinesin in moving vesicles around the cell. Traces of two kinesin molecules along a microtubule are illustrated in Fig. B. Consider these data and answer **all** the following questions.



1. (4 pts) What supplies the free energy needed for unidirectional (non-Brownian) movement along the microtubule?

2. (6 pts) What is the average rate of kinesin movement and what is the average step length?

3. (8 pts) In each protofilament the tubulin subunits repeat at 8 nm intervals. Given the average step length and this repeat interval, postulate a detailed mechanism for the movement of kinesin.

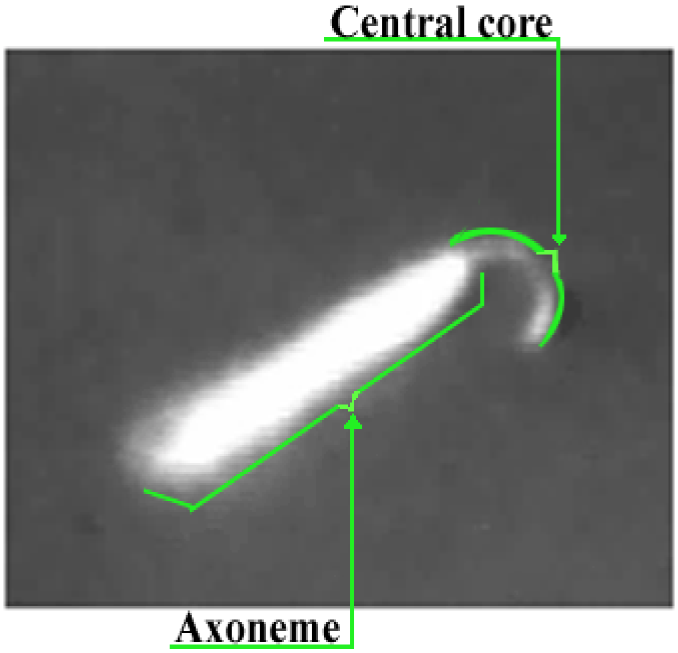
4. (4 pts) How many ATP molecules are likely hydrolyzed per step? How did you arrive at your answer?

5. (8 pts) Towards which end of the microtubule does kinesin move, and what biochemical constraints might polarize this movement? (Recall that dynein moves along MT in the opposite direction.)

Video Problem

3. The whip-like structure and motility of sperm and algal flagellae are complex and the relationship of structure to function is poorly understood. To investigate these phenomena, scientists have successfully removed intact flagellae from the cell bodies and then manipulated them in various ways, including stripping them of their plasma membrane envelopes. These isolated *axonemes* can then be further dissected with proteolytic enzymes and their behavior observed with high resolution, dark-field microscopy and recorded with an image-intensified digital camera. When the media is manipulated appropriately these modified axonemes continue to beat, but with time the axoneme core becomes extruded and the beating of the entire axoneme slows and stops. One such preparation is depicted below in the labeled figure on the left, which shows a single axoneme with its thinner central core extruded and slightly curled (from the right-hand end). The blunt base of the axoneme (on the left) is attached to the glass slide.

Double-click the figure to bring up the video and examine the motion of the axoneme and its displaced core. Assuming the behavior of this *in vitro* preparation resembles the more normal motion of the intact flagellum *in vivo*, answer the questions posed in the right-hand frame below.



[hyperlink here to <omoto3brotaxoneme.mov>]

Questions

1. What is the flagellar axoneme? Illustrate your description with a well-labeled, cross-sectional view, which indicates which component(s) form the "central core."

2. What chemicals must be present to elicit motility in this preparation?

3. How are flagellae thought to move? Briefly describe the mechanism and provide one piece of experimental data in its support.

4. What aspects of flagellar motility are currently not well understood or not adequately explained by the mechanism you described above?

5. Carefully describe the axonmene and core behavior in the video. Based on your answers to the first two questions above, hypothesize how the core behavior might contribute to flagellar movement.

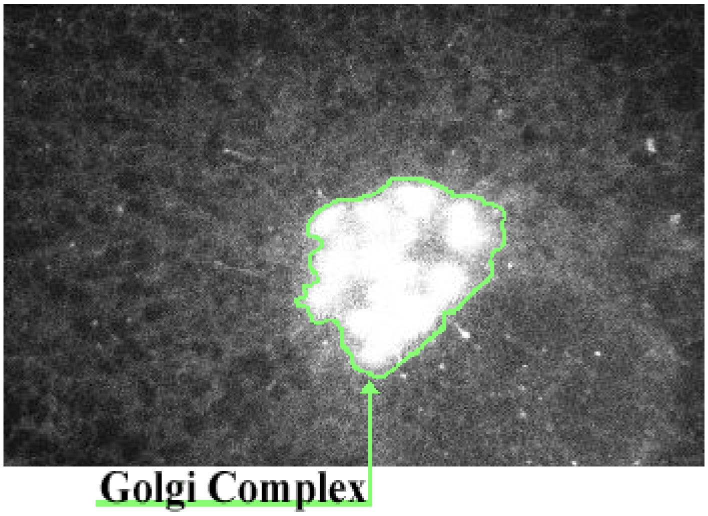
[You can find out more about the mechanism of eukaryotic flagellar movement in Omoto, *et al.* 1999. **Mol. Biol. Cell.** 10:1-4. PMID9880321 (PubMed ID number)]

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

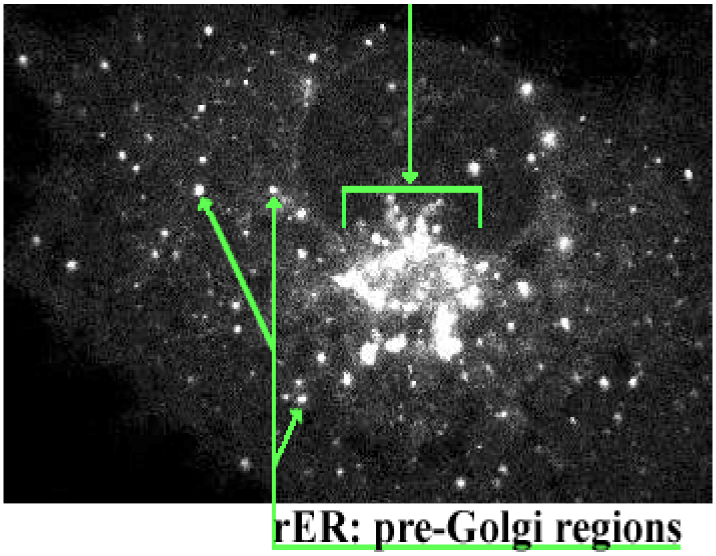
A. Describe carefully the movement of GFP-VSVP with respect to the Golgi complex.

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.

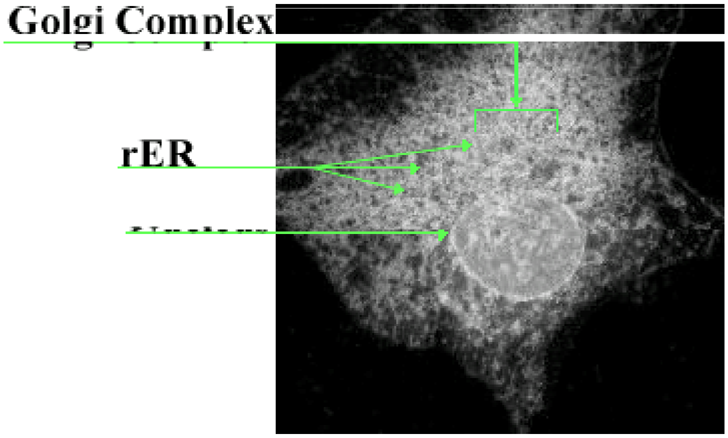
B. How do your observations compare with those made from watching the first video?

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?