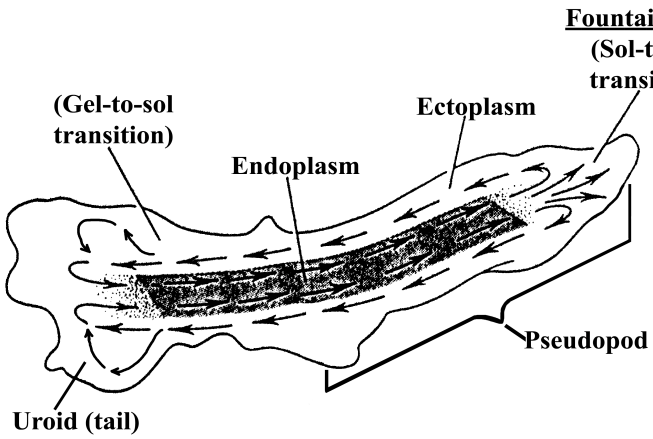
1. (28 pts) *Amoeba proteus*, a single-celled eukaryote, moves by means of psudopods attaching to and detaching from the substratum. Locomotion seems to be correlated with the forward flow of fluid cytoplasm (endoplasm) into an advancing pseudopod through a surrounding, gel-like ectoplasmic tube. The 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.	

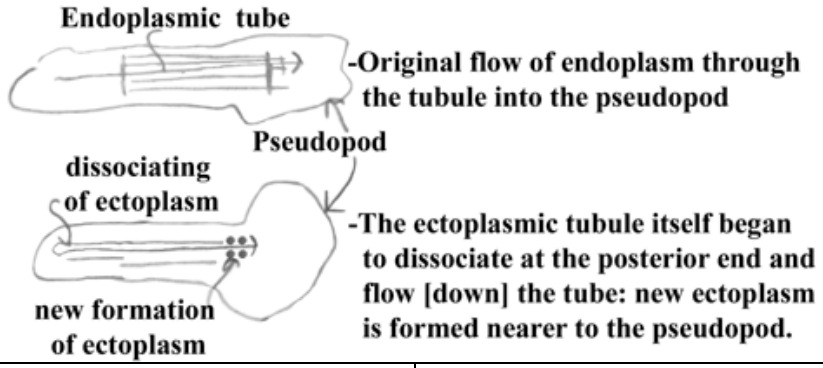
The validity of these two mechanisms were tested, respectively, in the following two examples of answers to **part C**. What do you think of them, as applicable to the two answers and more generally?

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	

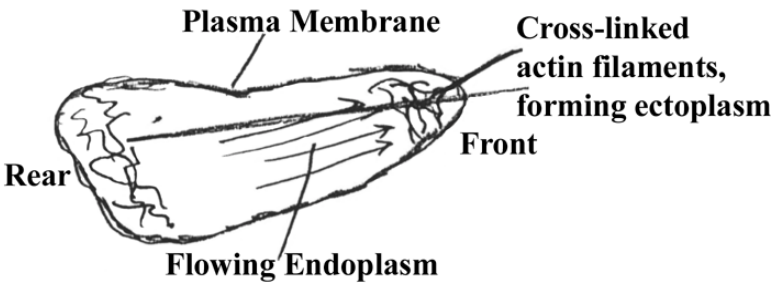
on the ratio of G-actin-ATP/G-actin-ADP subunits. If much G-actin-ADP was introduced into the system, say by microinjection, right after the formation of a new *A. proteus,* the concentration of G-actin-ATP would be so low that polymerization into microfilaments should be greatly inhibited. If this is 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.	

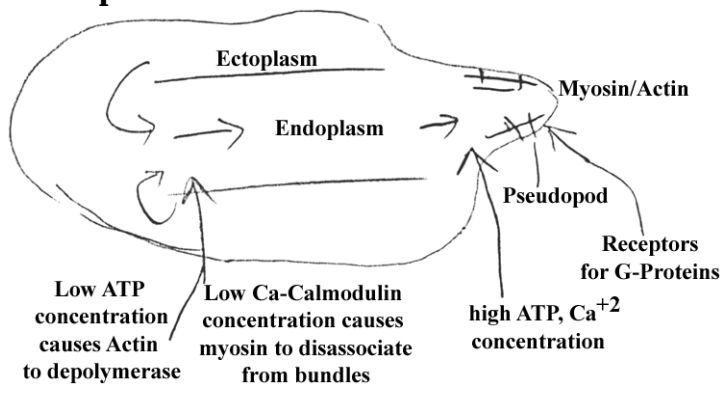
Question D. asks you to relate streaming *within* a pseudopod to locomotion of the whole cell across the substratum. Critique the two answers that follow and note in particular whether the qualities of the essays and diagrams are at all correlated. **Note** also an important feature of locomotion is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

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



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.	**Comments?**

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.	