INSTRUCTIONS

This is a 1 week long, open-book, take-home exam consisting of 4 multipart questions. We will put a pdf version of the exam on the website (in the lecture note section) in case anyone loses the hard copy.

Please read and follow these instructions carefully.

- ***PLEASE DO NOT DISCUSS THE QUESTIONS OR ANSWERS WITH ANYONE.*** *You are expected to generate the answers on your own with open access to any written resources. Unlike with the proposal, discussion with classmates, instructors, labmates, or anyone else is not allowed. If you need clarifications or have questions, email jais@u.washington.edu and bthielen@u.washington.edu. Jais will answer your questions by emailing the whole class, so that everyone has access to the same information.* ***We request that you email us your questions rather than asking us about in person, so that we can share the answer or discussion with the entire class.***

- ***Please read the questions carefully****. It is very easy to generate the wrong answer because you did not read the question carefully. Approach the answers logically; and don't make them harder than they are. Note that there are sometimes multiple correct answers to these questions.*

- ***You should not need to go to the primary literature in order to answer the questions.*** *The questions only require an understanding of textbook material, lecture notes, and assigned papers as well as discussions and issues in class. These questions emphasize integrating data and experimental design with your knowledge of material covered in class. For some parts of the exam a quick but brief literature search could make you more confident of your answer. But if you find yourself spending hours searching the literature, you are likely on the wrong track.*

- ***Answers should be typed, 12-point, single-spaced, and printed****. You do not need to include the questions; just label your answers 1A, 1B, 1C, etc. Approximate length for each answer is specified to help guide you with your answer. However, note that less is generally better, so be concise! Diagrams are encouraged and in some cases required, and can be handwritten or digital, but must be legible.*

- ***Instead of putting your name on the examination, use the last four digits of your student identification number.*** *This will allow us to grade your examinations blindly to ensure fairness.*

- ***The Last 4 digits of your student ID number should be on ALL pages, and pages should be numbered. Do NOT put your name on your exam.***

- *The mid-term is due on Thursday February 5th, at the START of class.* ***Please turn in a typed hard copy of the answers. Do not email your exams to us.***

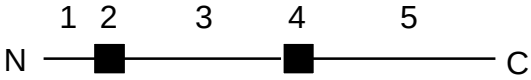
- *The total number of points in this exam = 100 (30% of final grade).*

ANSWERS ARE IN BLUE ITALICS BELOW EACH QUESTION (with some commentary on where people had trouble and the thought process we had hoped you would follow).

Midterm Question 1 (25 points)

Your lab is studying a new herpesvirus. By means of a screen, you recently found that viral gene P is necessary for your virus to replicate *in vivo* in an animal model but is dispensable in cell culture, and you wish to further characterize this gene. When you examine the predicted amino acid sequence, you observe two hydrophobic regions (2 and 4, see Q1 Fig. 1, length of segments are drawn to scale). To understand the function of the protein, you first want to determine its localization in the cell.

Q1 Fig. 1:



1A) Describe an experiment that you could perform to determine the localization of P in the cell, including a description of important controls. Assume you have successfully cloned the gene for P and you have a rabbit polyclonal antiserum that recognizes the P protein (One paragraph, 5 points)

All of you came up with one of the two experimental approaches I thought of to determine localization: immunofluorescence or immunogold electron microscopy. I accepted answers in which you mentioned looking at either P expressed by itself off a plasmid or in the context of a viral infection. There were two key types of controls that I wanted you to describe: 1) markers for different organelles (e.g. ER, Golgi, lysosome, PM) and 2) controls for antibody specificity (uninfected cells, secondary antibody alone). As we discussed in class when we read the papers about HIV budding in macrophages, individual markers may have their drawbacks, but when characterizing a new protein, it is important to have something with which to compare it.

Your preliminary studies suggest that the P protein is expressed in the ER. You examine the sequence and find a motif that you hypothesize may be important for its localization to the ER.

1B) What motif would you look for and how would you predict it would function? (One or two sentences, 2 points)

As we discussed in class, I was looking for an ER retention signal for this question (KKXX since this is likely to be a transmembrane protein due to its two hydrophobic segments). The ER retention signal interacts with cellular machinery mediating retrograde transport through the secretory pathway (Cop-1 coat protein). Just mentioning "signal sequence" is incorrect for this question because it alone would result in localization to the plasma membrane. Since I told you that the protein contains two hydrophobic domains, I wanted you to conclude that these will likely function as start & stop transfer sequences to direct the protein into the secretory pathway. Some people specifically mentioned this in their answers, but I did not require it. See pages 769-771 for a description of ER retention signals.

1C) How would you test whether this motif is *necessary* for ER localization? Describe an experiment you would perform and how you would interpret your findings (One paragraph, 4 points)

For this section, I wanted you to create mutants of P (either delete the retention signal or mutate it via site-directed mutagenesis) and then determine its localization as in part A. If the putative

ER retention signal is necessary, mutating it will result in its being relocalized (to the PM). If you said "signal sequence" for part B, I sill gave full credit for describing the same type of experiment (i.e. mutating or deletion the SS) in that context.

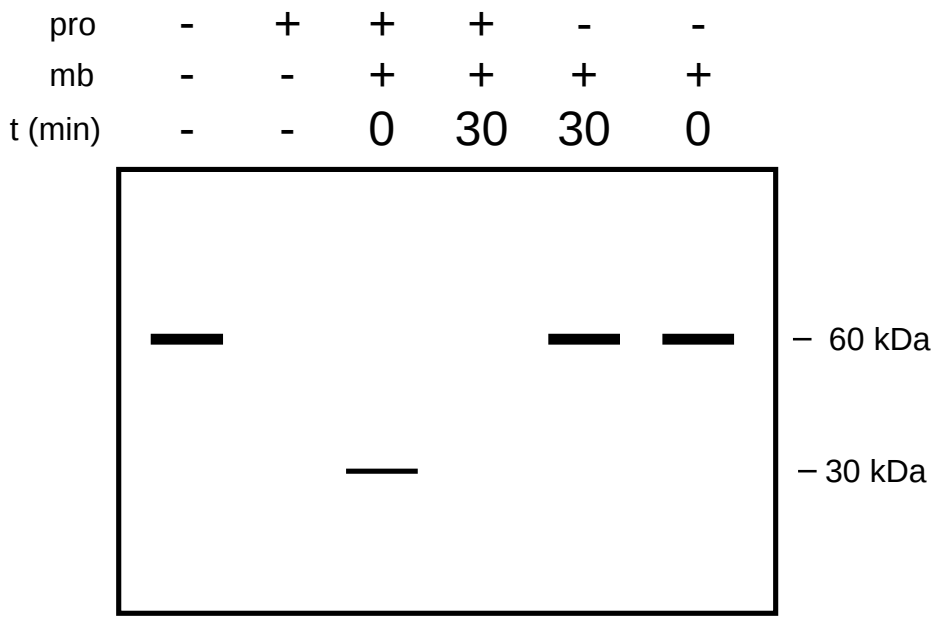
1D) How would you test whether this motif is *sufficient* for ER localization? How would this differ from testing whether it is necessary? (One paragraph, 4 points)

For this section, I wanted you to describe placing the putative ER retention signal on another protein that doesn't normally localize to the ER and then determining its localization. If it is sufficient for ER retention, adding the ER retention motif alone will redirect the protein. As one of you astutely pointed out, the ER retention signal will only be sufficient if you put it on a protein that already gets into the secretory pathway via a signal sequence, e.g. a plasma membrane protein. I also wanted you to describe the difference between necessary and sufficient. Necessary tells you that a motif is required for localization but may be one of two or more motifs within the protein that contribute. Sufficient tells you that it is the only motif within the protein required to confer localization. I was not looking for anything about the cellular machinery that is necessary for transport. Again, I gave full credit if you discussed this in terms of the signal sequence. I did not give credit for cell-free translation approaches because these can only tell you about translocation into the ER, not ER localization. The CFS does not include organelles such as the Golgi or plasma membrane so it is not an appropriate system for examining cellular localization.

Given its localization to the ER and its role during *in vivo* infection, you hypothesize that the protein may be involved inhibiting translocation of viral peptides across the ER membrane for antigen presentation (more about this later in the course). To study this, you decide to develop an *in vitro* system for studying protein P at membranes. In your first experiment, you use a "cell-free system" to translate P in the presence of ^{35}S methionine and analyze the translated material by SDS-PAGE followed by autoradiography. The results are shown below in Q1 Fig. 2:

Q1 Fig. 2

mb = ER membranes
t (min) = interval between initiation of translation and addition of membranes
pro = protease added at the end of translation reaction



1E) Based on these data, draw a cartoon illustrating the topology of P relative to the ER membrane. Be sure to clearly label both the ER lumen and cytosol in your diagram. (cartoon, 5 points)

I was looking for a drawing in which segments 1 and 5 were drawn in the cytosol, segments 2 and 4 span the lipid bilayer, and segment 3 is in the ER lumen. (See Fig. 12-48). If you look at Fig 2, you will see that there is no change in size between lane 1, which lacks membranes, and lane 6, which contains membranes at the start of translation. Therefore, you know that start transfer sequence is not cleaved, and your drawing should contain all segments 1-5. You know that segment 3 is in the lumen because protease treatment only generates a single protein product about half the size of the full length protein. If the orientation were reversed (i.e. 1 and 5 are in the lumen), you would see two different-sized fragments in lane 3. Pages 734-736 in the textbook explain translocation of multipass membrane proteins.

1F) In the course of your studies, you generate a form of P that no longer contains the motif important for ER localization. Assuming no other specific sorting signals, where would this version of P localize when expressed in cells and why? Draw a cartoon to illustrate this, again clearly labeling appropriate compartments. (One or two sentences + cartoon, 5 points)

For this section, I wanted you to say that removing the ER retention signal would result in relocalization to the PM. Segments 1 and 5 would remain in the cytosol and segment 3 would be in the extracellular space. As we discussed in class, the default for proteins that enter the secretory pathway is the cell surface, either PM if the protein has transmembrane domain(s) or secreted if it does not. Separate sorting signals are necessary for it to be retained in organelles such as the ER, Golgi or lysosomes. I gave partial credit if you said cytosol after giving "signal sequence" as an answer in part B.

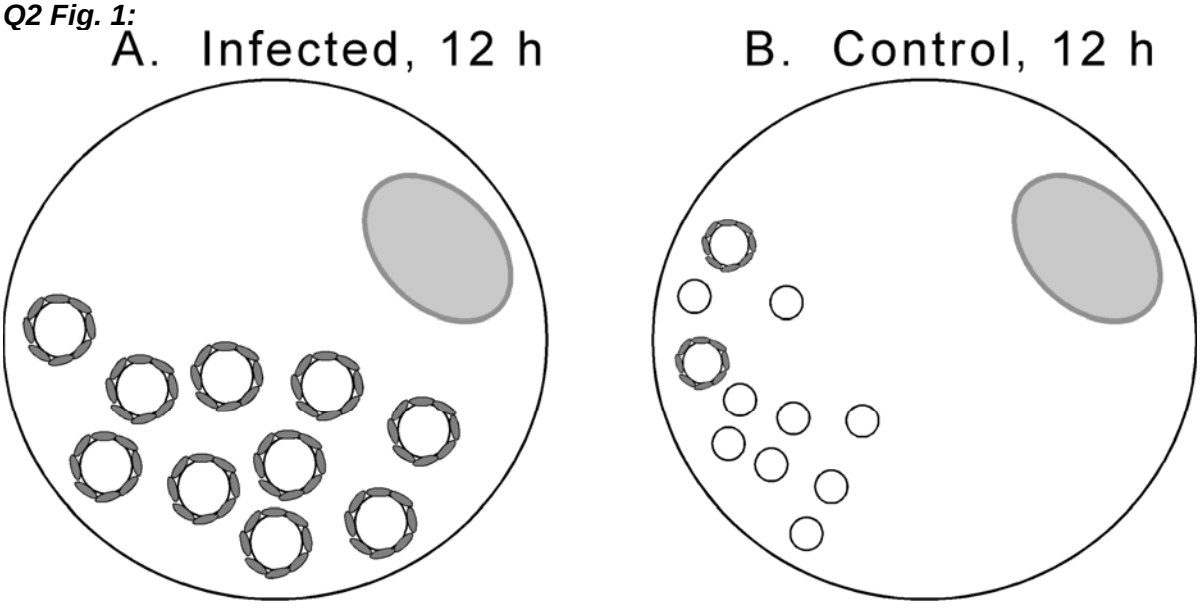
Midterm Question 2 (25 points):

You are a new postdoc in a lab studying a recently identified bacterial pathogen, *Studentis sufferus*. This organism is able to remain hidden inside host cells, eventually causing significant cell death. How the bacterium survives inside the cell is not known; nor is it known how it causes cell death.

The postdoc who worked on this organism before you demonstrated that when *S. sufferus* first contacts a mammalian cell, it uses a type III secretion system to inject the a toxin (Suffero toxin) into the host cell. (David Sherman will be lecturing soon on bacterial secretion mechanisms like type III secretion systems; however, for the purposes of this question you simply need to know that the bacterium makes a syringe like apparatus that allows it to inject bacterial protein effectors into the cytoplasm of the host cell.) After injecting Suffero toxin into the host epithelial cell, the bacterium is endocytosed through a clathrin-mediated pathway.

Your project is to figure out what the Suffero toxin does. You start by using transmission electron microscopy with negative staining to examine mammalian epithelial cells at different times after infection with *S. sufferus*. What you see is shown in Q2 Fig. 1:

Q2 Fig. 1:



You conclude that endocytic vesicles in the infected cell are larger but also morphologically abnormal. You have Suffero toxin encoded on a plasmid, so you decide to transfect cells with vector alone versus Suffero toxin plasmid. You see that Suffero toxin alone gives you the same pattern as in Q2 Fig. 1A (while vector alone gives you the pattern seen in Q2 Fig. 1B). You also have an antiserum to Suffero toxin, so you use that to confirm that the toxin is expressed upon transfection.

2A) Propose a hypothesis for what Suffero toxin does based on the results of your experiments. (One or two sentences, 3 points).

Hypothesis: Suffero toxin prevents disassembly of clathrin coat proteins on clathrin coated endocytic vesicles. (Most people received full credit for this. If you keep in mind the basic principle that vesicle coats go through cycles of assembly and disassembly, the most likely possibility is that these coats are stuck in the assembled form,)

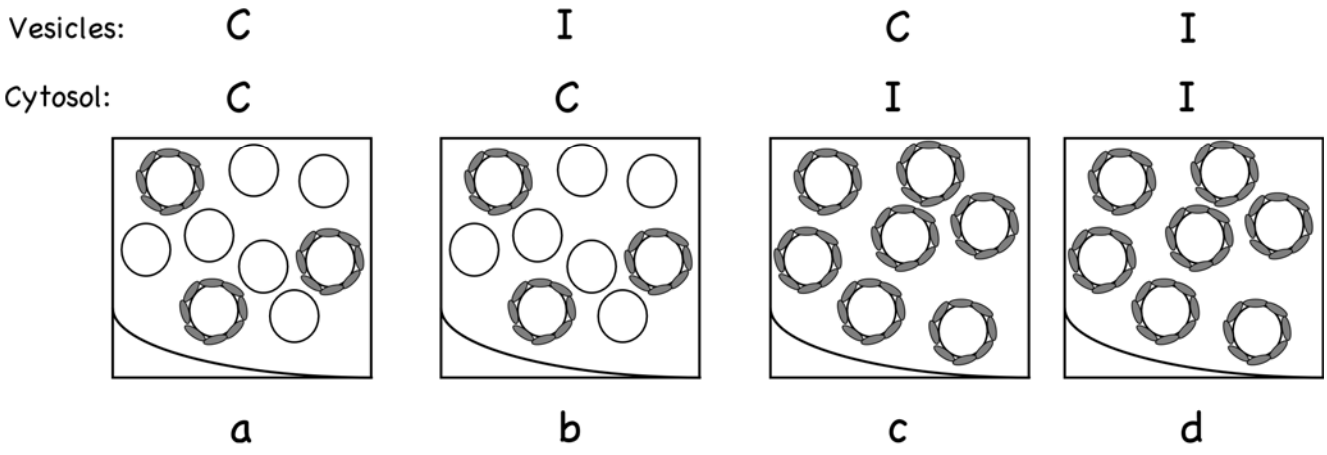
You perform the following experiment:
You isolate endocytic vesicles from control cells and infected cells. You also isolate cytosol from control cells (C) and infected (I) cells. You mix vesicles from control cells with cytosol from control vs. infected cells; you also incubate vesicles from infected cells with cytosol from control vs. infected cells.

2B) What question will this experiment test? (One sentence, 3 points)

Whether Suffero toxin acts on a factor in the cytosol or whether it acts on a factor attached to vesicles. (Most people received full credit for this. Some people lost a little credit if they did not say what the two possibilities are. This is important to keep in mind – most well designed experiments have at least two possible outcomes and you should think through how you would interpret results for both possibilities.)

When you perform this experiment, you obtain the results shown in Q2 Fig. 2:

Q2 Fig. 2:



2C) What do you conclude from the data in Q2 Fig. 2? (one short paragraph, 3 points)

If you mix cytosol from infected cells with vesicles from normal cells, the coats fail to disassemble. But if you mix vesicles from infected cells with cytosol from normal cells, the vesicles disassemble normally. Therefore Suffero toxin appears to act on a factor in the cytosol

that causes accumulation of coats on endocytic vesicles. (Most people received full credit for this.)

2D) Given these findings and assuming that Suffero toxin acts on only ONE factor, state which factor you think Suffero toxin acts on and describe how your model explains the observed results. (one or two paragraphs, 5 points)

Since the vesicles from infected cells appear fairly normal, most likely Suffero toxin acts on something that is important for coat disassembly but is located entirely in the cytoplasm. The most likely candidate would be Arf, the regulatory GTPase. The toxin could cause Arf in the cytoplasm to be locked in a GTP bound state. This would allow coats to be assembled but would not allow them to be disassembled which requires GTP hydrolysis.

Other acceptable answers include:
1) Suffero toxin could inactivate a GAP that acts on Arf. Since GAPs hydrolyse GTP, inactivation of the GAP that acts on Arf would cause the clathrin coat to stay locked onto vesicles.
2) Other cytoplasmic factors that are credibly involved in coat disassembly. Many people found evidence of other regulatory factors in the literature. However, if you invoke another factor, you needed to at least mention Arf as a possibility or describe how Arf works during disassembly as part of your answer to show that you considered this key player in coat disassembly. The reason for this is because you should always consider the simplest possibilities before you jump to invoking complex scenarios (the principle of parsimony, which is an important one in science.) Or put another way, always consider the basics before you start considering the "bells and whistles".
Those of you who didn't think about Arf would likely have thought about it if you had reviewed the lecture notes on vesicle coats and thought about the basic molecular machinery involved in coat formation described in the lecture.

2E) Explain how your model for Suffero toxin action explains how S. sufferus evades host defenses (two or three sentences, 3 points).

If the bacteria entered into endocytic vesicles before the toxin acted, then the bacteria would have a means to evade degradation, since the vesicles affected by the toxin cannot fuse to lysosomes because they cannot disassemble their coats. (Almost everyone received full credit for this.)

2F) Describe two additional experiments that will BEST test your model. Both experiments should utilize the general experimental set up described in Fig. 2 and tools that you either know you have or that are expected to be commercially available (i.e. don't propose to use complicated approaches). EXPLAIN EXACTLY HOW YOU WILL INTERPRET YOUR EXPERIMENTS. (Less than half a page, 6 points).

Best experiment: titrate out the toxin by expressing a large excess of Arf (or Arf-GAP). One way you could do this is by transfecting cells with Arf or Arf-GAP plasmid (or vector control). Confirm they are making a large excess of Arf (or Arf-GAP), and then infect with a defined small dose of S. sufferus. Cells that are overexpressing Arf (or Arf-GAP) should have fewer vesicles with locked coats, because there should be a limited amount of toxin and an excess of Arf (or Arf-GAP) available. The Arf (or Arf-GAP) excess should be able to bind it toxin but still leave enough left over to act normally to help with disassembly (assuming that there isn't a preference for the inactivated Arf to bind to vesicle coats).

Many people received full credit because they provided a relatively "definitive' experiment like the one described above. People who described experiments that are only "suggestive" lost a few points.

Before setting up your experiment in Fig. 2, you performed some controls to make sure your purified endosomes and lysates were functioning properly. In one of these experiments, you added a large excess of a non-hydrolyzable analog of GTP. This analog binds properly, but cannot be hydrolyzed to GDP because of a modification in its structure.

2G) How would you expect control vesicles incubated in control lysate to look after you incubate with the non-hydrolyzable GTP analog? (One sentence, 2 points)
They should look like vesicles in 2c and 2d. Those of you who were on the wrong track should have gotten a big clue from this answer. It should have made you think about the striking similarity between simply locking GTP in a non-hydrolyzable state and what is seen with the toxin. This would have helped you realize that a very simple and plausible mechanism for toxin action would be for the toxin to prevent GTP hydrolysis. Note that there is some conceptual similarity to cholera toxin which causes an enzyme to be "locked" in the "permanently on" form because regulatory mechanisms for turning it off are inactivated.

Those of you in Pathobiology who lost points because you didn't discuss or consider the Rab GTPase and its modulators should very carefully review this. While I am not a fan of Pabio's style of qualifying exam, when asked to contribute a question I frequently write one that asks about GTPases because I think they illustrate so many key concepts of regulatory machinery. So consider yourselves forewarned! ☺

Midterm Question 3 (35 points):

You are studying trafficking in cells infected by a newly identified viral pathogen that infects T cells. You have just obtained antisera against four of the proteins encoded in this virus, W, X, Y, and Z.

Your student validates the antisera. When he infects cells with the virus and performs indirect immunofluorescence on cells at 48 hours using a primary antibody against W, he finds Protein W localized at the plasma membrane. Your student makes tagged constructs and examines their localization. When he puts either an HA or myc tag on the N terminal end of Protein W and expresses tagged W in the context of the whole viral genome, he finds W in the cytoplasm. However, when he puts either an HA or myc tag on the C-terminus of protein W and expresses that in the context of the whole viral genome, proper plasma membrane localization occurs. Your student concludes that the protein must be an integral membrane protein.

3A) What was the student's reasoning in coming to this conclusion based on his experimental findings? (One paragraph, 3 points)
The student reasoned that because the tag at the N-terminus prevents proper localization, there must be an important determinant of localization at the N-terminus. Since the protein is localized to the plasma membrane, the important N-terminal determinant could be a signal sequence, in which case this could be an integral membrane protein. The signal sequence would cause the protein to be cotranslationally translocated; transmembrane segments later in the protein would cause it to be an integral membrane protein.

(While this is in fact one possibility the flaw in this thinking is that there are alternate mechanisms for trafficking to the plasma membrane via an N terminal signal.)

Note that an integral membrane protein is a protein that fully spans the membrane; in contrast to an acylated or GPI anchored protein which is inserted into only one leaflet of the lipid bilayer.

To see if his conclusion makes sense, you go back to the sequence for protein W predicted from the cDNA and observe that the first 15 amino acids starting with the initiating methionine are predicted to be:

MGARKKRLNSKVIRD

3B) Based on the sequence information, do you agree with your student's conclusion? What information in the sequence supports your answer? (One paragraph, 4 points)
If the student's hypothesis was right, you should have found a hydrophobic N terminus since signal sequences are typically hydrophobic. Instead what you find is a strikingly hydrophilic N terminus with a lot of basic charge. So that argues against a N terminal signal sequence and should make you think about other signals for targeting proteins to membranes.

To help resolve this controversy, you ask your student to make two additional constructs. The first consists of the first 10 amino acids (shown above) fused onto the N terminus of GFP. Your student finds that adding these 10 amino acids alone to GFP causes GFP to localize to the plasma membrane.

3C) Given these data and the sequence of the N terminus, what is your hypothesis about how protein W traffics to the membrane (be specific)? What aspects of the data support your hypothesis? (On paragraph, 5 points)
The signal shown here is a myrisotylation signal. Myristate is attached co-translationally to a glycine at position 2 (which is indeed present in the sequence for Protein W). Moreover, myristoylation typically acts with basic residues that facilitate binding to acidic phospholipids heads in the membrane. So your hypothesis should be that this protein is translated in the cytoplasm, is cotranslationally myristoylated, and targets to the plasma membrane via an N terminal myristate.

Need to say myristoylation and mention glycine at position 2 and basic residues to get full credit. You don't get credit if you think this is happening via ER translocation or any other organelle.

3D) You ask your student to make one more mutant to more definitively test your hypothesis? What is the mutant you want him to make? (One sentence, 4 points)

Mutate the glycine (i.e. to alanine) and show that you no longer get targeting to the plasma membrane.

Until now, your student has always expressed W along with all the whole virus. Interestingly, when he expresses W alone without any tag, he finds it localized in the cytoplasm. He hypothesizes that another viral protein is needed for plasma membrane targeting. So he systematically tests this hypothesis using constructs with deletions and demonstrates that expression of X is required to target W to the plasma membrane.

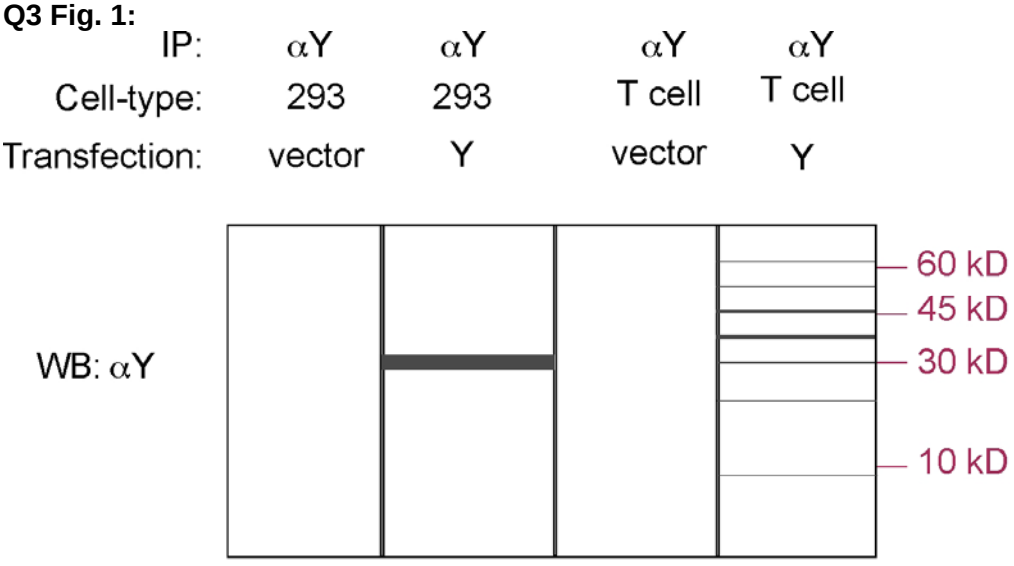
3E) What is your hypothesis for how X changes that topology of W, based on these data? (One paragraph, 5 points)

Myristates are frequently buried and need to be exposed through conformational changes (see lecture notes). Given the data it is likely that X alters the conformation of W (probably by binding it, but other mechanisms can be envisioned), causing the myristate to be exposed and target W to the membrane.
To get full credit you need to mention that a conformational change is likely needed to expose the myristate and only occurs in the presence of X.
Some people proposed exposure of the myristate signal so that N-myristoyl transferase (NMT) could put myristate onto it. Since myristoylation is usually thought to be cotranslational, this is less likely but I gave people some credit for this since you may not have picked up on that detail.
Others thought that X might be NMT itself; that is highly unlikely since NMT is present in eukaryotic cells and therefore does not need to be encoded by the virus.

Another student in your lab is studying protein Y. She finds that when protein Y is expressed in a 293 cells (epithelial-derived human kidney cell), it is localized to the ER by indirect immunofluorescence with an anti-Y antibody. However, when it is expressed in a human T cell, it is localized primarily in the cytoplasm.

You ask her to repeat the experiment, denature the lysate, and immunoprecipitate Y with anti Y antibody from the denatured, diluted cell lysate. She separates proteins the eluted proteins by SDS-PAGE, transfers to nitrocellulose, and western blots with anti-Y antibody expecting to see the 30 kD Y protein. Q3Fig. 1 shows the result of that experiment.

Q3 Fig. 1:



3F) Taking into account what is known about mechanisms for how viruses evade hosts, generate a hypothesis for how Y is trafficked to the cytoplasm in T cells. Your hypothesis should take into account all the data shown in Q3Fig. 1. (One paragraph, 6 points)

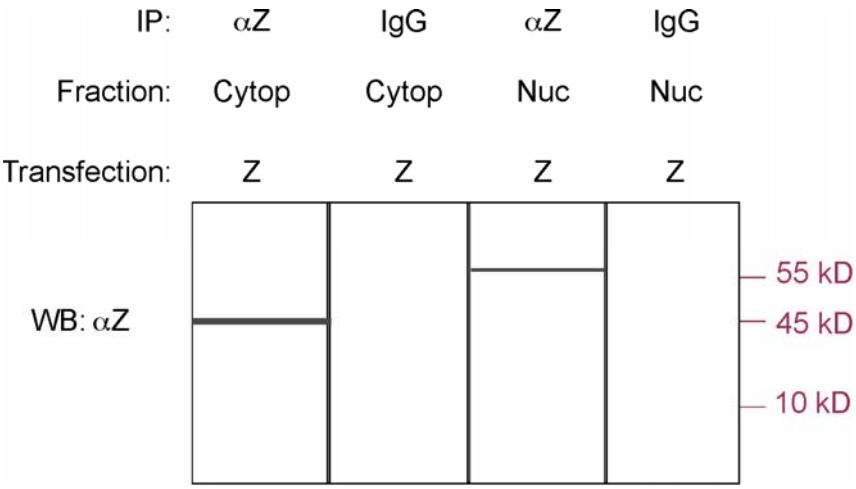
In T cells, Y is retrotranslocated out of the ER and is multiubiquitinated (which accounts for the ladder of higher bands in T cells, each one ~8 kD bigger than the one below it). Once in the cytoplasm it is degraded, accounting for the smaller bands seen on the gels.

3G) Speculate on why the Protein Y might undergo the trafficking that you described in your answer to 3F (i.e. what might be going on that could be beneficial to the virus?). (One paragraph, 3 points)

Most likely, when Y retrotranslocates, it is taking a cellular protein involved in the host immune response (such as MHC class I or II) with it and causing that protein to be degraded (along with Y). This is a mechanism known to be used by CMV (see lecture 2 CMV US11 retrotranslocates MHC class I out of the ER into the cytoplasm for degradation).

This student is also studying Protein Z. When Z is deleted from the virus, no viral replication occurs, so Z appears to be essential. Z is present in the cytoplasm and in the nucleus. Your student transfects Z into 293 cells, separates nuclei and cells by centrifugation, denatures each fraction, and performs an immunoprecipitation on each fraction using anti-Z antibody or normal rabbit IgG. Q3Fig. 2 shows the result of her experiment. In addition, she demonstrates in a separate experiment that the N-terminal half of protein Z localizes to the nucleus and cytoplasm, as is the case for wild-type Z, but the C-terminal half is localized only to the cytoplasm.

Q3Fig. 2:



3H) Present a hypothesis for trafficking of Protein Z into the nucleus that takes account of all the data for Protein Z and known mechanisms of nuclear trafficking. (One paragraph, 5 points)

Most likely, Protein Z has a nuclear localization signal in its C terminus. However, this signal is only exposed after the protein is post-translationally modified. This is all you need to say to get full credit.

In fact, the modification is a sumoylation. Sumo groups are like ubiquitins but are a little larger (10 – 12kD) and frequently regulate nuclear transport. We didn't cover this directly in class but referred to it briefly in our discussion of ubiquitin like modifiers (like pupylation). You did not need to realize this is sumoylation to get full credit.. However, the modification is much too

large for a phosphorylation (if it was phosphorylation it would have to be many phosphorylations). If you said it was a phosphorylation and did not grapple with the size issue, then you did lose a little credit. Ubiquitination would have been a much more reasonable modification to propose.

Midterm Question 4 (15 points):

You have a friend who is studying RNA expression of the virus described in Question 3. The virus makes 4 spliced transcripts for expression of early genes, and one unspliced full-length genomic RNA that is used for expression of late genes (which are not encoded on the spliced transcripts). Your friend tells you about three new pieces of data:

1) He has discovered that the unspliced transcript is not expressed if the 100 bp at the 3 prime end of the full length RNA (called region A) is deleted.

2) He also encoded stop codons into various viral genes and discovered that expression of one early protein, E1, is required for expression of all the late proteins.

3) Leptomycin B has no effect on expression of either early or late viral genes.

Comments from Jais on this question: I designed RNA expression of this virus to be exactly like HIV except for one difference -- it does not use the Crm1 pathway. So in many ways it was simply a "compare and contrast" question, but was more challenging because it was given to you as data.

4A) What controls do you want to make sure your friend has done when he tells you the results of the Leptomycin B experiment? (One or two sentences, 5 points).
He should include a control to make sure his Leptomycin B is working (stock is active, concentration is correct, etc.), i.e. show that U snRNA's or HIV genomic RNA or a protein that utilizes the Crm1 pathway is not exported when he adds Leptomycin B. One person realized that the easiest way to do this control would be to test the export of a GFP-labeled protein that uses the Crm1 export pathway. It is much easier experimentally to score export of a GFP-labeled protein rather than scoring export of RNAs.

4B) Describe a model for export of spliced and unspliced RNA for this virus. Your model should be the simplest one that is consistent with current thinking about RNA export and also explains all of the data. For all trafficking events in your model, state whether you hypothesize that a specific trafficking pathway is used and what your evidence is for the putative pathway. If you lack enough information to speculate about a pathway, indicate that as well. (One or two paragraphs, 5 points).
The 3 prime end of the full length RNA (region A) likely contains an RNA export element that is required for export. The virus likely bypasses cellular retention mechanisms by encoding viral protein E1, which likely binds to the RNA export element and facilitates its export through a cellular export pathway. So E1 is analogous to the HIV Rev protein and region A is like the Rev response element in HIV RNA. We do not have enough information to know what pathway is used for export of the full length unspliced RNA. However, we do know that the Crm1 pathway is not used because of the Leptomycin data (see lecture 3). It would be reasonable to hypothesize that the TAP pathway is since this is the typical export pathway for mRNAs.

4C) What is the ONE MOST DEFINITIVE experiment that your friend could perform to identify (or confirm) which nuclear export pathway is used by the unspliced genomic RNA? (One or two sentences, 5 points)

Note that I asked for an experiment that would DEFINE or CONFIRM the export pathway used by unspliced genomic RNA. There is a big difference between definitive experiments and suggestive ones. To get full credit you had to design a definitive experiment, i.e. knockdown of the hypothesized critical export factor such as TAP. Dominant negative constructs are also relatively definitive. Someone suggested an antibody that would bind TAP; that is on the right track but very difficult technically (would require injections of cells which is much less standard than siRNA knockdowns).