Spring 2003 Molecular Biology Exam #2 – Applying Lessons

There is no time limit on this test, though I have tried to design one that you should be able to complete within 4 hours, except for typing. You are not allowed to use your notes, any books, any electronic sources except those specified in the exam, nor are you allowed to discuss the test with anyone until Monday March 24, 2003. **EXAMS ARE DUE AT 11:30 ON MONDAY, MARCH 24**. You may use a calculator and/or ruler. The answers to the questions must be typed on a separate sheet of paper unless the question specifically says to write the answer in the space provided. If you do not write your answers on the appropriate pages, I may not find them unless you have indicated where the answers are.

For the figures, I took photographs from journals, so you may detect warps or angles that seem odd. This is due to my need to reduce glare and shoot form about a 45 degree angle. Do not take this odd perspective into consideration for your answers. Ignore the tilted angles of the figures.

When you are ready to take the exam, send me an email with the subject line of **Molecular Test**. This will generate an automated email telling you how to download the exam.

-3 Pts if you do not follow this direction:
Please do not write or type your name on any page other than this cover page. Staple all your pages (INCLUDING THE TEST PAGES) together when finished with the exam.

Name (please print here):

Write out the full pledge and sign:

Here is the honor code
http://www.davidson.edu/student/redbook/honorgeneral.html#honorcode

"On my honor I have neither given nor received unauthorized information regarding this work, I have followed and will continue to observe all regulations regarding it, and I am unaware of any violation of the Honor Code by others."

Average = 88% range from 100 – 70%

How long did this exam take you to complete (excluding typing)?

6 pts.

1. Using the information below, tell me how to make:

a. 300 mL solution of 1.5M NaCl, 50mM EDTA, 0.25M Tris pH 7.3.

26.3 g NaCl

6.24 g EDTA

9.07 g Tris

Dissolve in about 200 mL water, pH to 7.3 with HCl, then bring final volume to 300 mL.

b. 50mL of a 0.8% w/v agarose gel that is 0.75X TBE.

0.4 g agarose

3.76 mL 10X TBE

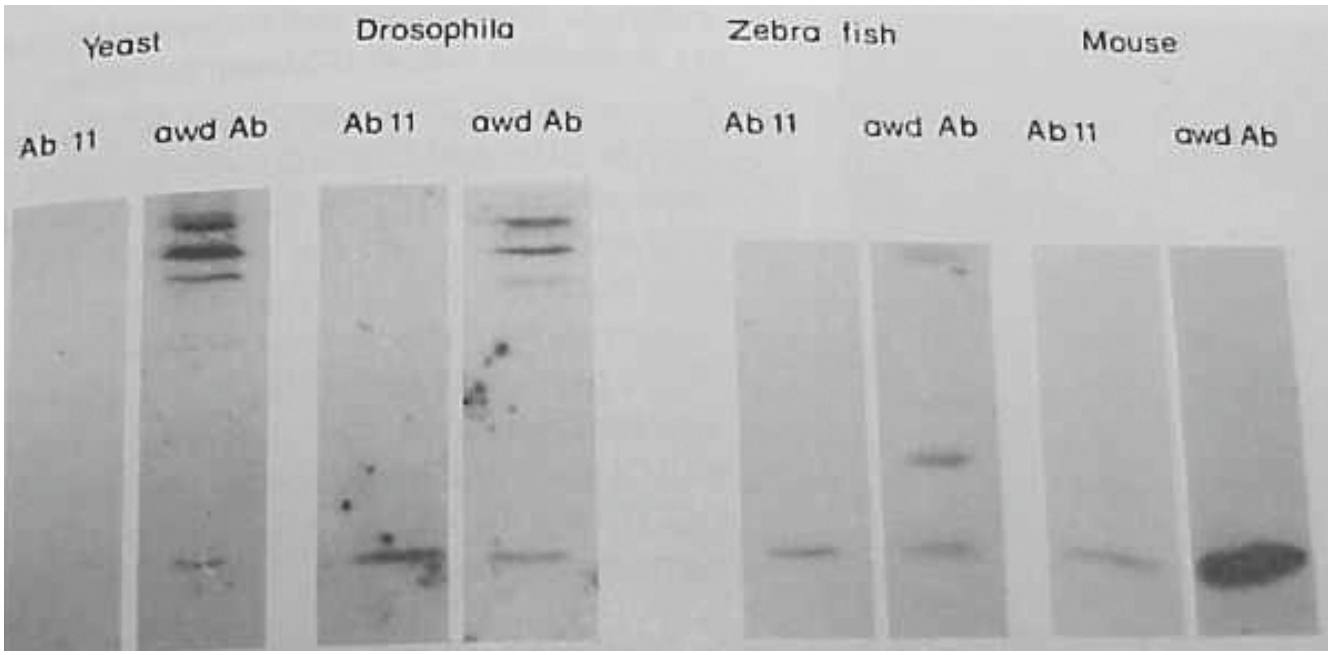
46.24 mL water

microwave to melt, cool, and pour.

FWs: NaCl = 58.5; EtBr = 394; EDTA = 416; Tris = 121; HCl = 36.5; agarose = 204. Other raw materials include SDS = stock solution of 20%; TBE = stock solution that is 10X;

10 pts.

2.



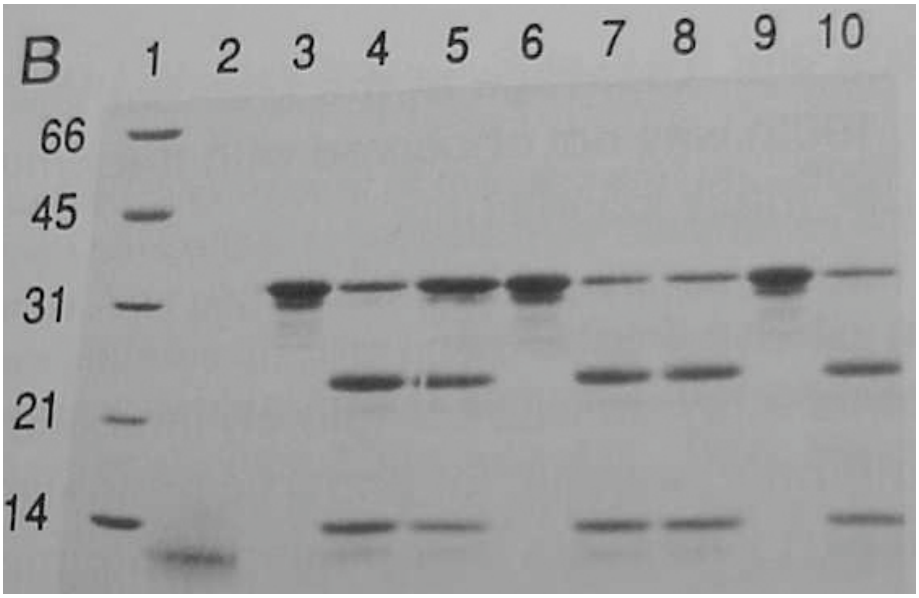
Two antibodies were made against a fly protein that we'll call **Wow**. The first antibody was called "Ab 11" and the second antibody was called "awd Ab". The investigators used these two antibodies to probe western blots made from tissues of different species as labeled in the figure. Interpret these data as fully as you can.

Your answer needed to contain the following information:

Fly Wow must be the small MW protein since this is the only band the two antibodies have in common. The three band at the top might be artifact, except similar bands are seen in yeast. Perhaps these represent proteins of similar sequence. Both Ab11 and awd Ab work in fly.
Yeast has Wow and the 3 similar proteins. However, the more specific antibody Ab11 does not recognize the yeast Wow. This shows for sure that the two antibodies bind to different epitopes.
Fish has Wow, one f the 3 upper bands and a new middle-sized band too. Both antibodies work in fish.
Mouse has only the Wow protein or at least the other 3 have different sequences and are not recognized by awd Ab. The band is very intense in mouse but not with Ab11. This may indicate alternative splicing that is recognized by awd but not Ab11. However, it appears that the Wow protein is more conserved across all species.

10 pts.
3. A group of authors, including 2002 Nobel laureate Paul Nurse, wrote in 1988 that yeast *S. pombe* produces a protein called cdc2 which is a kinase and has a molecular weight of about 34 kDa. In *Xenopus* frogs, a similar function was found in a heterodimer complex called MPF. The purified MPF subunits were 32 and 45 kDa in molecular weight. Starting with these 2 frog proteins, tell me how you could experimentally determine which *Xenopus* protein was the ortholog of the *S. pombe* protein and how you could clone both the yeast and the frog orthologous cDNAs.
I was thinking a functional test would be best. However, many of you went straight for sequence comparisons, which is fine. However, you had to describe a logical way to isolate the frog cDNA.

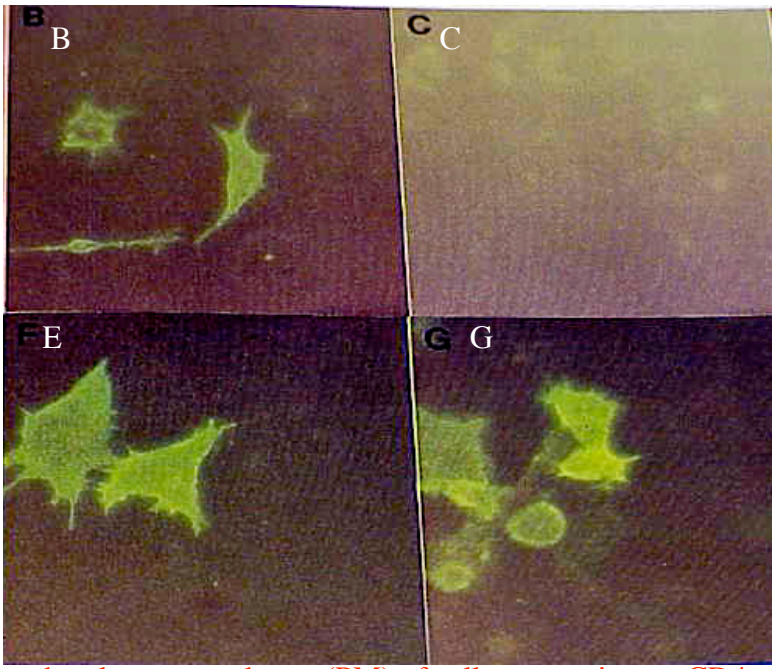
14 pts.
4. In this figure, you are seeing an SDS-PAGE stained with a Coomassie blue (a protein stain). Lane 1 = MW markers; lane 2 = HIV protease; 3 = generic protein called PRC; 4 = PRC + HIV protease; 5 = PRC + HIV protease + 0.01 mM protease inhibitor called pepstatin A; 6 = PRC + HIV protease + 0.1 mM pepstatin A; 7 = PRC + HIV protease + 1.0 mM protease inhibitor antipain; 8 = PRC + HIV protease + 1.0 mM protease inhibitor leupeptin; 9 = PRC + HIV protease + 0.02% w/v SDS; 10 = PRC + HIV protease + 5 mM EDTA. Interpret these data as fully as you can.



1. MW marker
2. protease about 12 kDa in MW.
3. protein of about 33 kDa. Shadow band may be degradation product from normal breakdown.
4. HIV protease produces 2 new bands 24 kDa and 14 kDa. Some 33 kDa protein is undigested. Protease can be seen faintly at about 12 kDa.
5. Inhibitor reduces protease activity a little – less 14 kDa band and more 33 kDa band.
6. Pepstatin completely inhibits protease at this higher concentration.
7 – 8. Theese inhibitors do not inhibit protease even at 10X higher concentrations.
9. SDS denatures protease so it cannot cleave protein.
10. EDTA binds divalent cations so this protease does not require cations to work.
This looks like a good way to find new protease inhibitors for HIV.

12 pts.

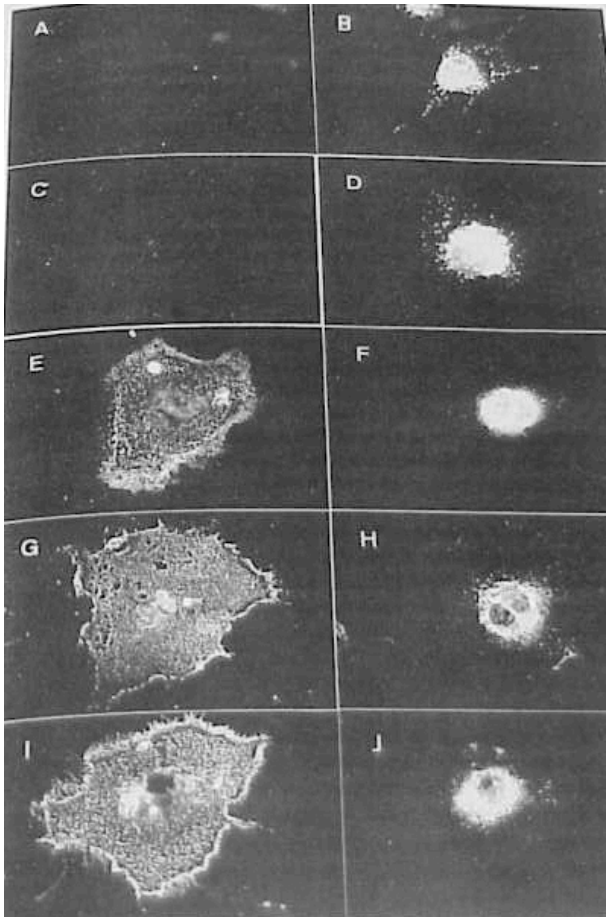
5. In this figure (next page), you see some COS cells transfected with variations of the T helper cell protein called CD4. Panels B and E show cells transfected with wt CD4 cDNA and labeled with fluorescent HIV viruses (B) or fluorescent anti-CD4 antibodies (E). Cells in panels C and G were transfected with a mutant CD4 cDNA that encodes two missense mutations (46 K→N and 47 G→V) in CD4. Cells in were labeled with fluorescent HIV viruses (C) or fluorescent anti-CD4 antibodies (G). Interpret these data as fully as you can.



B shows HIV binding to the plasma membrane (PM) of cells expressing wt CD4.
E shows wt CD4 expressed on the PM of cells.
C shows a lack of HIV binding to the mutant CD4. Could be due to lack of protein expression, but panel G shows the protein was made. Therefore, these two amino acids play a role in HIV binding. People with this allele might be resistant to HIV.

10 pts.

6. In this figure, you are seeing immunofluorescence data from a series of experiments trying to determine how a protein called the **invariant chain** (abbreviated **Ii**) is able to be retained in the ER. In the left column, the cells were labeled with red fluorescent primary antibodies before the cells were treated with detergent. In the second column, similar cells were treated with Triton X-100 before labeling with green fluorescent primary antibodies. In panels A and B, the cells were transfected with wt Ii cDNA, while all other panels show cDNA deletion mutations of Ii: C and D have the first 11 amino-terminal amino acids deleted; E and F have the first 15 amino acids deleted; G and H have the first 20 amino acids deleted; I and J have the first 23 amino acids deleted.



Interpret these data as completely as you can.

Triton opens all membranes so the antibodies (Ab) can access all areas. Thus, right column shows all proteins are being made and are in the ER.

A and C show no labeling because Ii is retained in the ER. Because Ii is wt, this is the normal behavior.

E-I show Ii is no longer retained in the ER. Ii is located in the PM of these cells.

So: Ii does not use KDEL for ER retention.

first 11 amino acids not necessary for retention.

amino acids 12-15 are necessary for retention.

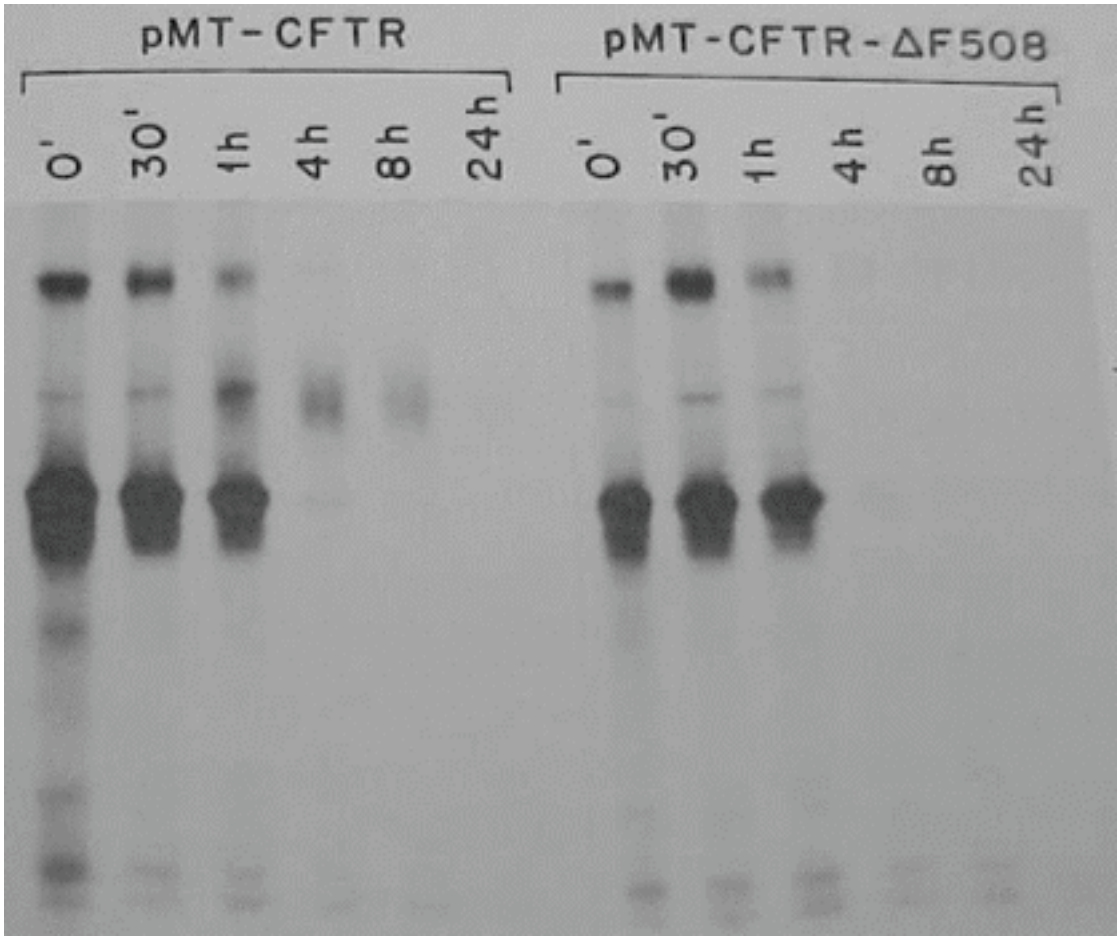
16 pts.

7. In this figure, You see the results of an SDS-PAGE followed by fluorography. All cells were transfected with a plasmid (called pMT-CFTR) containing *wt* CFTR cDNA (left side) or a plasmid (called pMT-CFTR–Δ508) containing mutant CFTR cDNA (right side). Cells were labeled with ^{35}S-methionine for 15 minutes and then the radioactive media was replaced with non-radioactive media for the times indicated at the top of the fluorograph. At the indicated times, cells were immunoprecipitated with an antibody that binds CFTR and the precipitated material was loaded onto the SDS-PAGE. Interpret the data as fully as you can. Do not cite data from question 8 for question #7. You may look at question 8, just don't incorporate those data into this answer.

CFTR–Δ508 is the most common allele for cystic fibrosis (CF). I was working on the assumption that this would sound familiar from Bio111 and would reinforce what you had learned once before. Regardless, you should have been able to determine:

CFTR is the band of greatest intensity. Pulse and no chase shows how much was made in wt and mutant CFTR. Chase shows what happens to these molecules over time. In one hour, not much changed. But by 4 hours, the wt CFTR appears as a smear of larger MW. This suggests it is

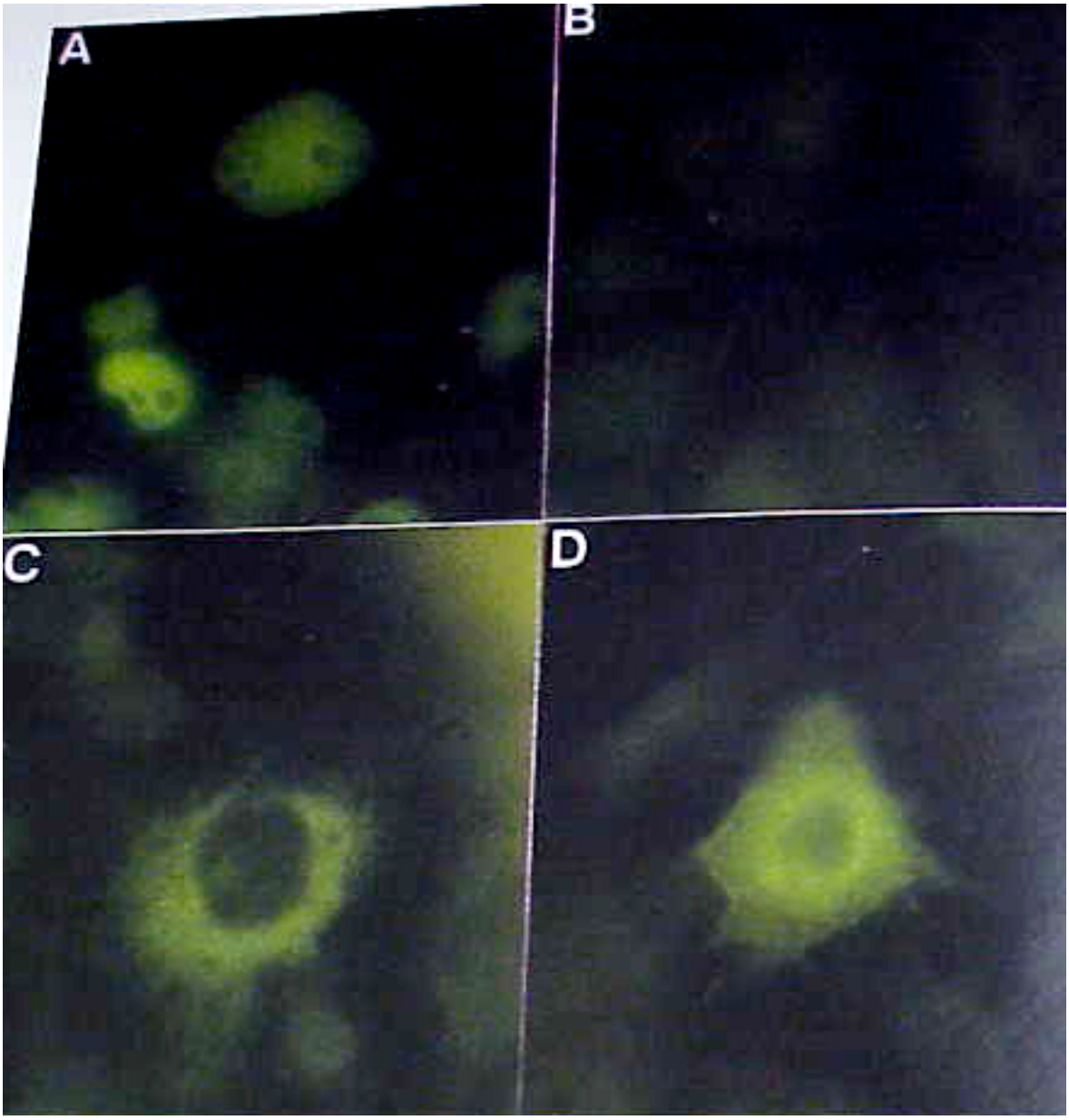
glycosylated on its way to the PM (question 8). CFTR–Δ508 does not get glycosylated and appears to be destroyed within the 4 hour period. This is a case where ER degradation is clearing the cells of mutant proteins.



12 pts.

8. The figure (next page) for this question is 4 immunofluorescence images which appeared as the next figure in the paper from question 7 above. In all four panels, COS-7 cells were transfected with cDNAs and then labeled with antibodies. Panel A shows mock transfection labeled with anti-SV40 large T antigen. Panel B shows cells transfected with *wt* CFTR and labeled with anti-CFTR but in the presence of excess CFTR added to the incubation. Panel C shows cells transfected with CFTR–Δ508 and labeled with anti-CFTR antibodies. Panel D shows cells transfected with *wt* CFTR and labeled with anti-CFTR. Interpret these data as completely as you can but do not cite data from question 7 in your answer. You may consider question 7, just don't incorporate those data into this answer.

This question did not work very well. I thought more people would remember CFTR and think of it sitting in the PM of cells. Therefore, I just graded for a description of what you saw. You got extra points if you interpreted the data as ER labeling in panel C and PM in panel D. Panel A shows the large T antigen (of SV40) which we have described in class is added to COS cells which enables them to be used for production of your favorite protein in transient transfections.

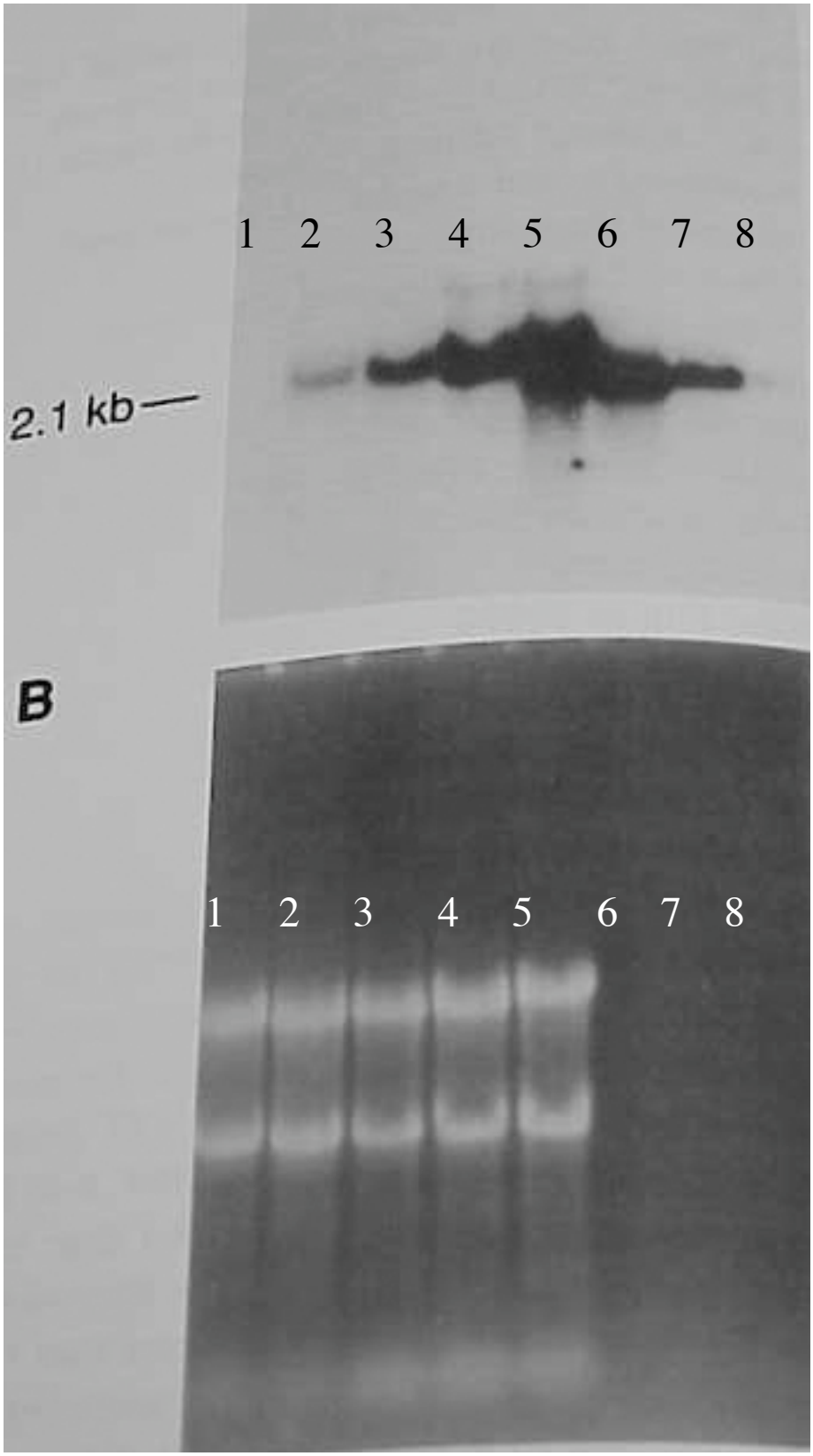


10 pts.

9. The data for this last question are on the next page. You see a Northern blot above a photograph of the gel used for the same Northern blot. The gel was soaked in ethidium bromide and then photographed under UV light. There are equal numbers of lanes in the blot and the gel, but don't worry about the sources of the materials for the gel.

Interpret this figure as completely as you can. Assume that the gel was loaded properly, no samples were lost and no samples spilled into unintended lanes. The intense bands in panel B are ribosomal bands since total RNA was loaded in lanes 1-5.

Since you were told the gel was used to produce the blot, there is no error in lanes etc. The key was that lanes 1-5 had total RNA but lanes 6-8 had other RNA (i.e. mRNA). The amounts of total RNA loaded in lanes 1-5 in the gel are about the same. So the level of the mRNA in the blot must be increasing from lanes 1 (undetectable) to 5. There is more mRNA in lane 6 than7, and no detectable amounts in lane 8.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