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:

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

6 pts.

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

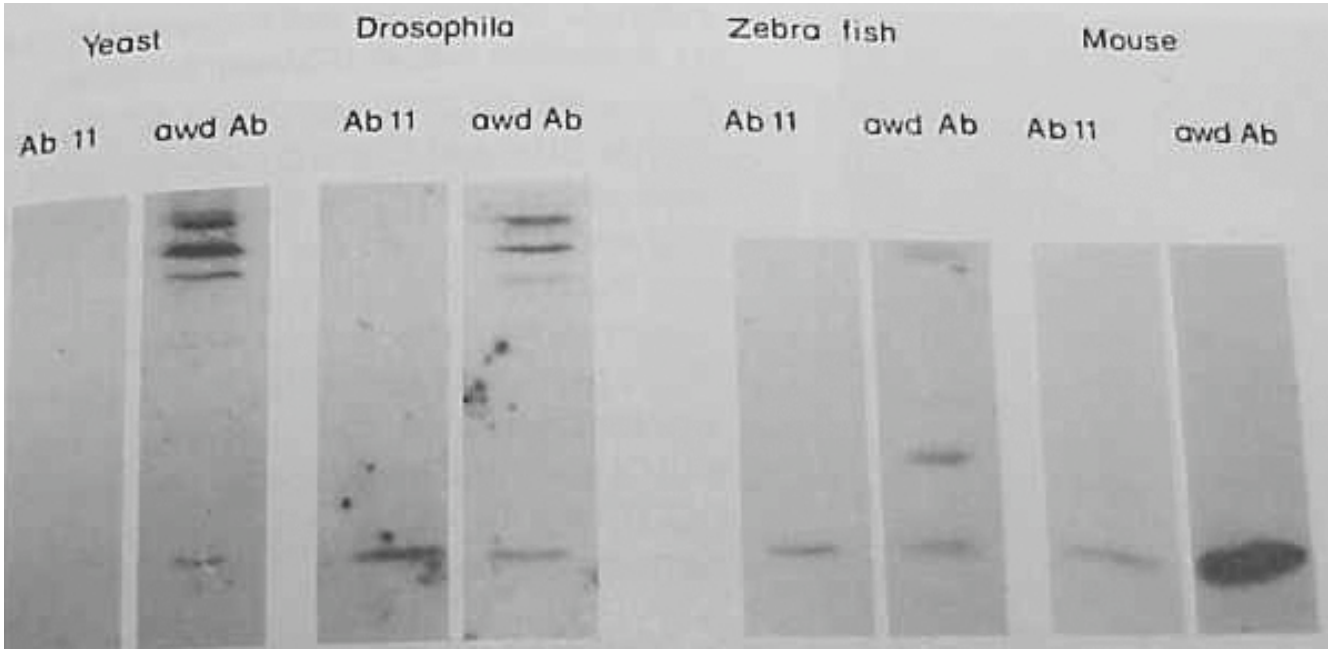
a. 300 mL solution of 1.5M NaCl, 50mM EDTA, 0.25M Tris pH 7.3.

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

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.



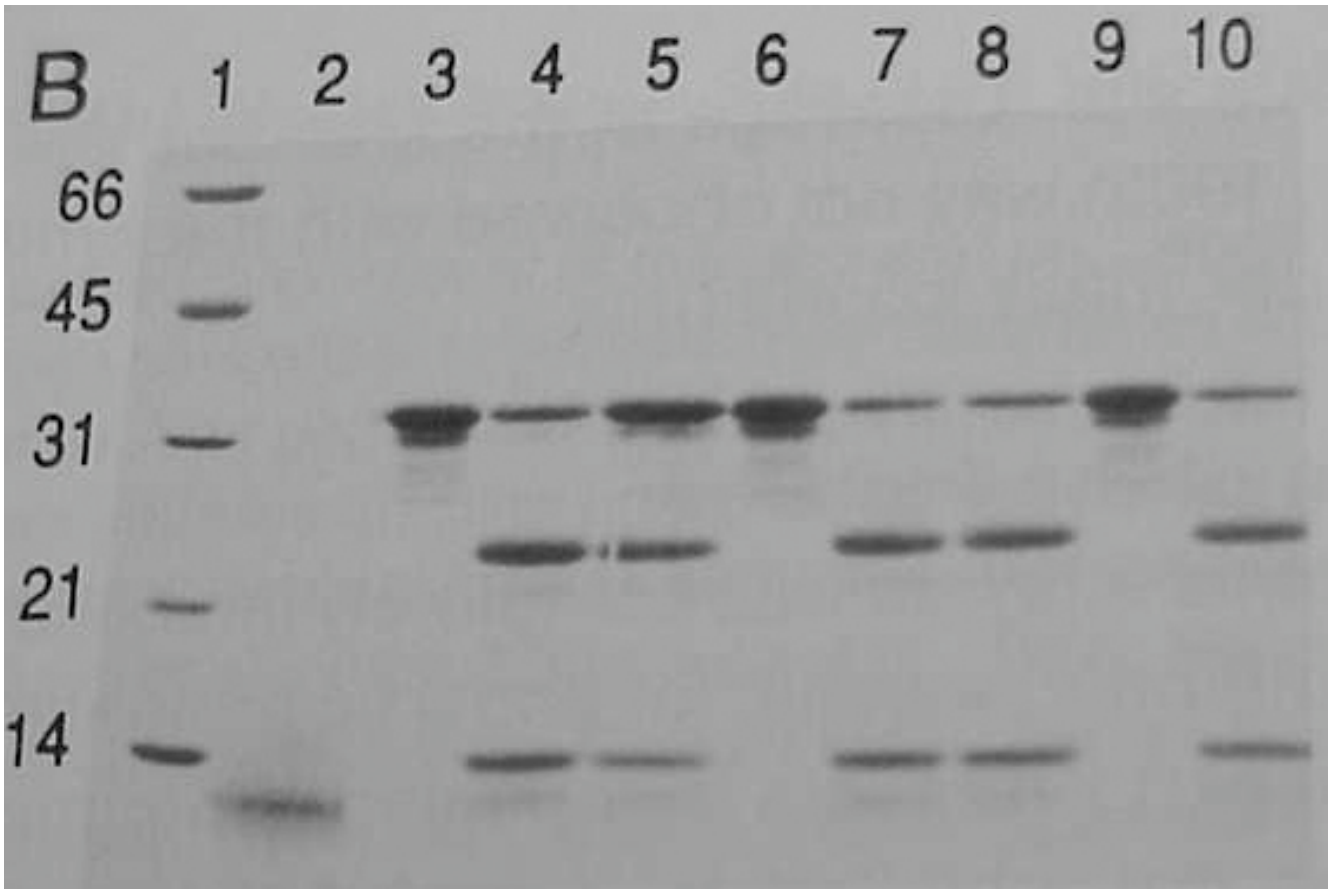
Two antibodies were made against a fly protein that we'll call **Wow**. The first antibody was called "Ab 11" and the second antibody was called "awd Ab". The investigators used these two antibodies to probe western blots made from tissues of different species as labeled in the figure. Interpret these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

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.

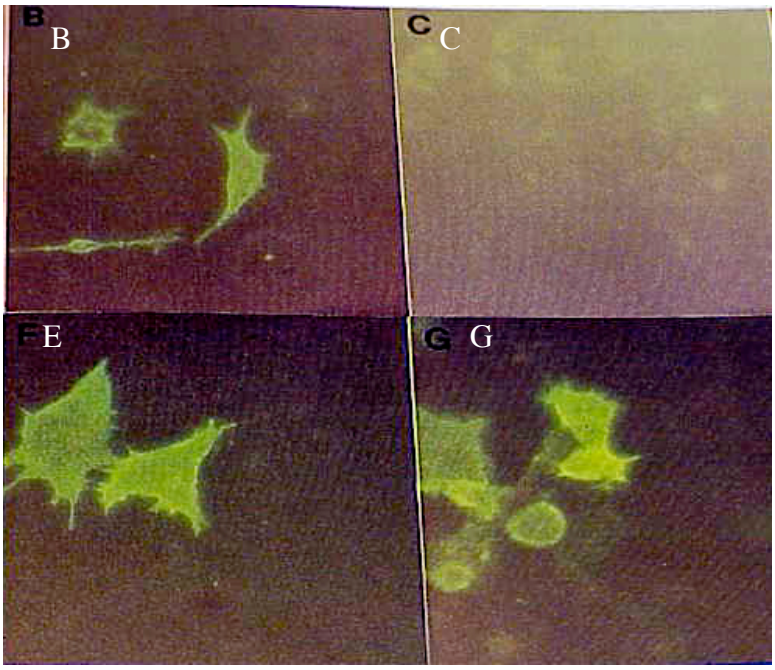
14 pts.

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.



12 pts.

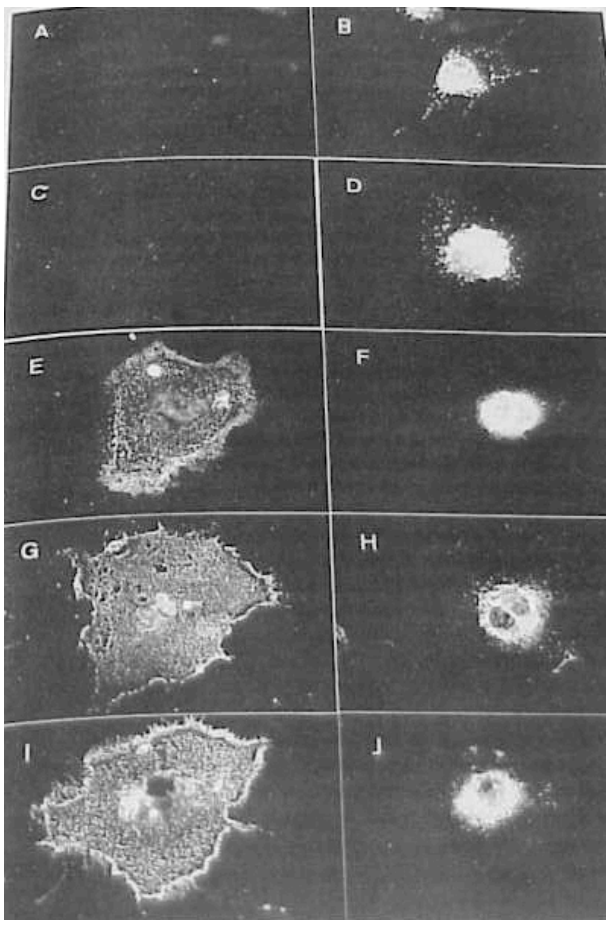
5. In this figure (next page), you see some COS cells transfected with variations of the T helper cell protein called CD4. Panels B and E show cells transfected with wt CD4 cDNA and labeled with fluorescent HIV viruses (B) or fluorescent anti-CD4 antibodies (E). Cells in panels C and G were transfected 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.



10 pts.

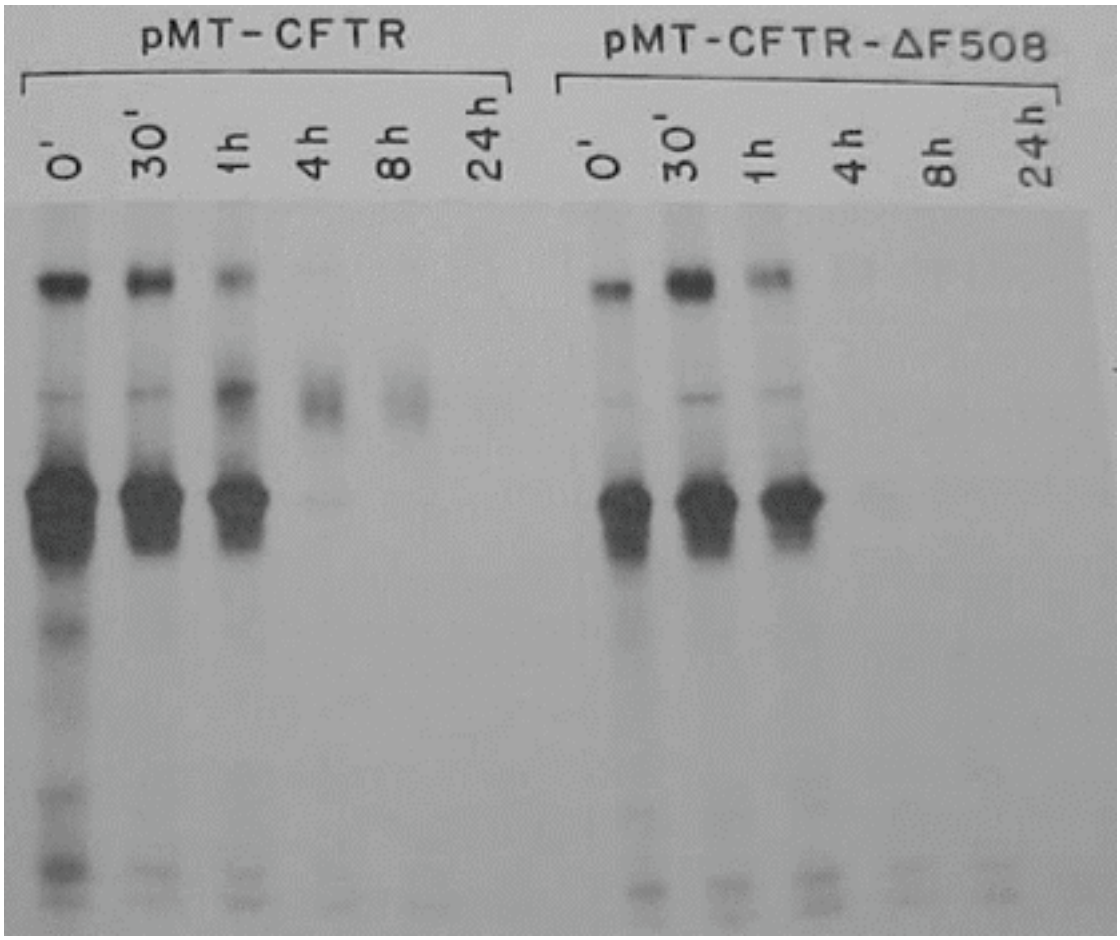
6. In this figure, you are seeing immunofluorescence data from a series of experiments trying to determine how a protein called the **invariant chain** (abbreviated **Ii**) is able to be retained in the ER. In the left column, the cells were labeled with red fluorescent primary antibodies before the 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.



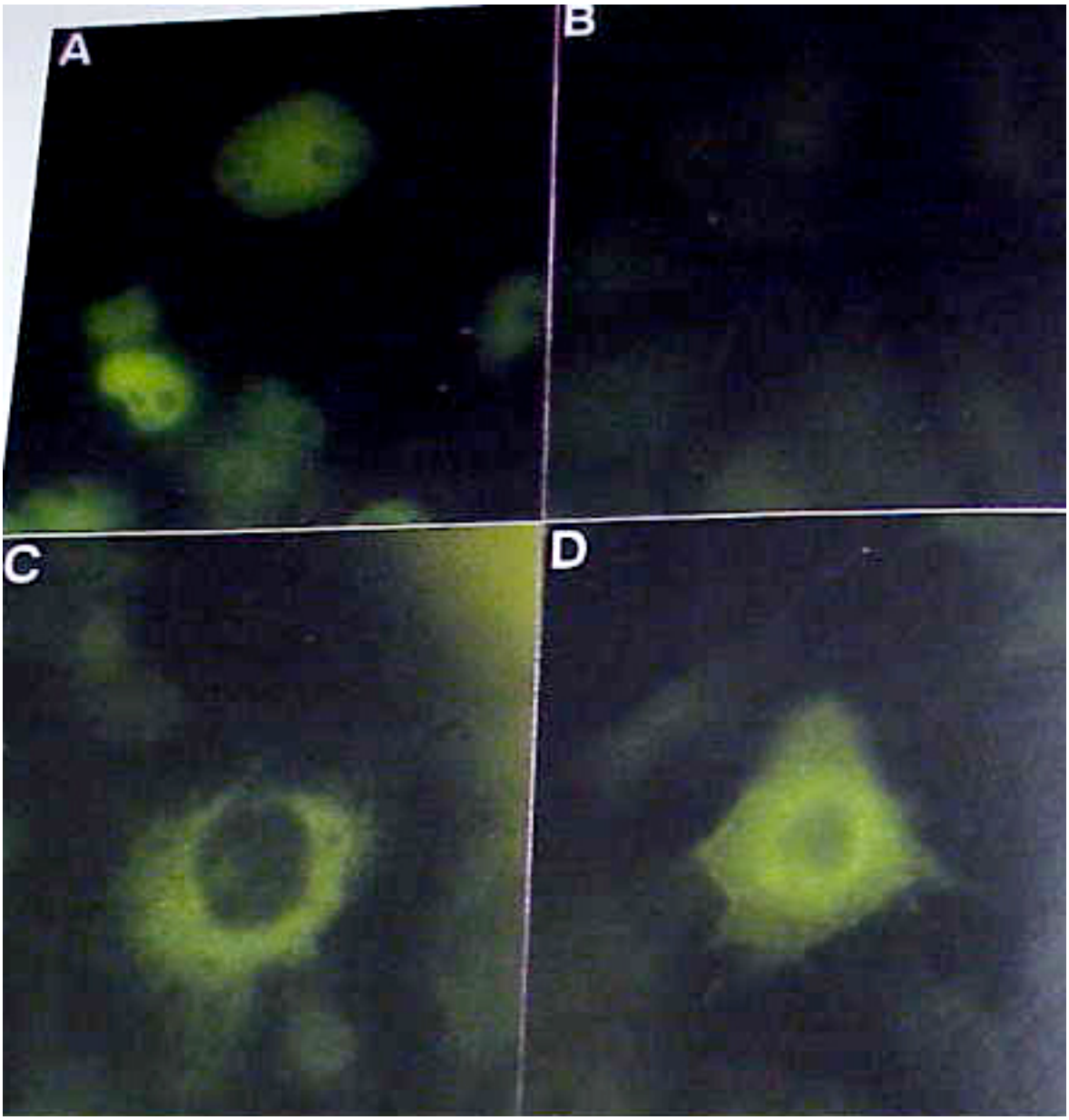
16 pts.

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–ΔF508) 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.



12 pts.

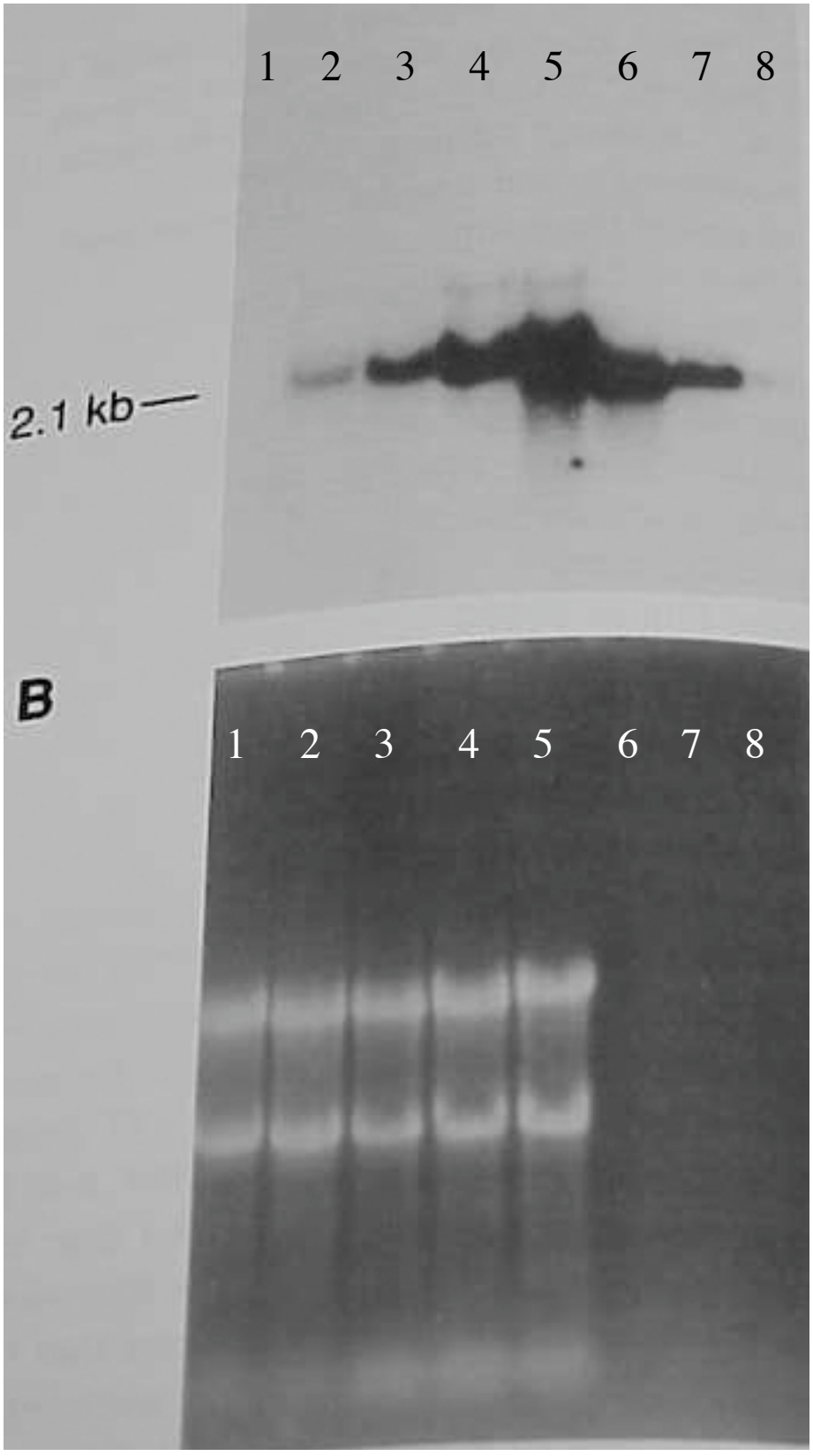
8. The figure (next page) for this question is 4 immunofluorescence images which appeared as the next figure in the paper from question 7 above. In all four panels, COS-7 cells were transfected with cDNAs and then labeled with antibodies. Panel A shows mock transfection labeled with anti-SV40 large T 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–ΔF508 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.



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.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