Original

Spring 2000 Molecular Final Exam - Using the Tools

There is no time limit on this test, though I have tried to design one that you should be able to complete within 3 hours, except for typing. You are not allowed to use your notes, or any books, any electronic sources, nor are you allowed to discuss the test with anyone until all exams are turned in at 9 AM on Monday May 15, 2000. **EXAMS ARE DUE AT 5 PM ON FRIDAY MAY 12**. 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.

-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)?

Mol. Exam #3

12 pts.

1) Tell me how to make the following solution.

Make 250 mL of 5 M NaCl, 1.5 M Tris-HCl pH 8.0, 6% w/v dry milk, 1.5% v/v antifoam A. (FW for NaCl = 58.44; FW for Tris = 121; FW for HCl = 36.5; FW for dry Milk = 77; FW for antifoam A = 100)

10 pts.

2) On the last page, there is an abstract that explains one process of allowing proteins to be imported into the ER while the proteins are being translated. Figure 1 goes with this abstract so you can see what the authors think is going on.

Describe in general terms how you would design experiments that you would need to perform to show the protein Sec61p is "essential and sufficient". Do not give me every detail, but don't be so brief as to say "add something, stir it, incubate, look at it".

60 pts. Obviously, this is an important question. Call me at work between 9 am and 5 pm if you do not understand something. Also write down any assumptions you are making.

3) This question has 4 parts to it. You need to know a bit about Stat1α which is a wild-type transcription factor that binds to the promoter part of the gene IRF-1 GAF. Stat1αs is a mutant allele that has serine #727 mutated (by site-directed mutagenesis) to alanine. You may go to this URL to look at the differences in serine and alanine structure <www.bio.davidson.edu/Biology/aatable.html>. You also need to know that when cells are exposed to IFN-γ, they turn on expression of Stat1α or Stat1αs (which every allele they have). "Preimmune serum" means polyclonal antiserum from a non-immunized animal.

A) Figure 2 shows a band-shift assay. Two sets of cells were use; for lanes 1-4, wild-type cells were used and for lanes 5-8, cells containing Stat1αs were used. Cells containing Stat1αs are homozygous and the only difference between the wild-type and mutant alleles is the single codon change. The probe DNA used is called IRF-1 GAF (or in the figure GAF for short) and is the promoter region of a gene called GAF (see general information above). The figure legend summarizes the different treatments. Interpret this figure fully.

B) Interpret Figure 3 from the same paper using similar experimental conditions as noted in the figure legend. Make sure you understand that two different antibodies are used in the top part of the figure - one that binds to both Stat1α and Stat1αs; the another antibody binds to any phosphorylated tyrosine. The bottom blot used a third antibody that bound to a different epitope present in both Stat1α and Stat1αs.

C) Figure 4 shows a bar graph summarizing some CAT assays using IRF-1 GAF promoter upstream of the CAT cDNA. Stat1β is another transcription factor that does not bind to IRF-1 GAF at all. Interpret this figure.

D) Hypothesize why the mutant allele does not behave the same as the wild-type allele in figure 4 (the only functional test). You must synthesize all the data in figures 2-4, plus the web page which is very important here.

Mol. Exam #3

12 pts.

4) Figure 5 shows you two models describing constructs for homologous recombination at two loci (HHO and MLH). Figure 6 shows some Southern blots (panel A) and northern blots (panel B). Demonstrate your homologous prowess by telling me which lanes are wild-type and which ones represent mutant lanes. You must do this for all four blots. **Put your answers here:**

A HHO probe
Lane 1 =

Lane 2 =

Lane 3 =

A MLH probe
Lane 1 =

Lane 2 =

Lane 3 =

B HHO probe
Lane 1 =

Lane 2 =

Lane 3 =

B MLH probe
Lane 1 =

Lane 2 =

Lane 3 =

6 pts.

5) Your final final exam question. Did the yeast IDP2 gene bind to the DNA of any species tested on your Southern blot? If so, which species?

Summary

We have analyzed early phases of the cotranslational transport of the secretory protein preprolactin through the mammalian endoplasmic reticulum (ER) membrane. Following recognition of the signal sequence of the nascent polypeptide chain in the cytosol by the SRP, the chain is transferred into the membrane, where a second signal sequence recognition step takes place for which the presence in the lipid bilayer of the Sec61p complex is essential and sufficient. This step leads to a tight junction between the ribosome-nascent chain complex and the Sec61p complex, and to the productive insertion of the nascent chain into the translocation site. These results show that a translocation substrate is subjected to two recognition events before being allowed to cross the ER membrane.

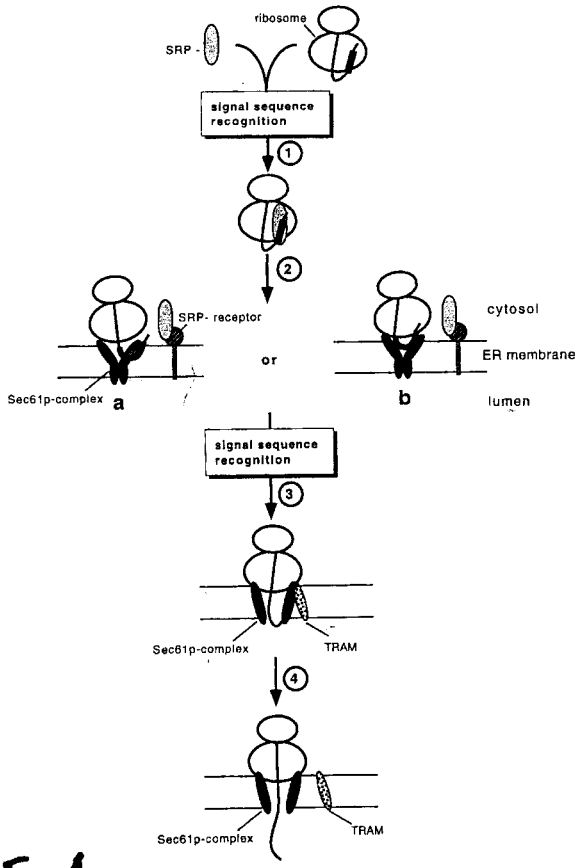


Fig 1

[redacted] Scheme of the First Steps in Cotranslational Protein Translocation across the ER Membrane

In step 1, SRP binds to the signal sequence of a nascent chain when it has emerged from the ribosome. In step 2, the complex of ribosome, nascent polypeptide, and SRP binds to the ER membrane, and the SRP is released from both the ribosome and the signal sequence. The ribosome binds to the Sec61p complex in a loose manner, and the nascent chain contacts Sec61α. At this point, the signal sequence may contact either both the Sec61p complex and the phospholipids (a) or only the protein (b). In step 3, the signal sequence is recognized in a process involving the Sec61p complex, and the nascent chain is productively inserted into the translocation site with its signal sequence contacting the TRAM protein. The ribosome becomes firmly bound to the Sec61p complex, and the translocation channel opens toward the lumen of the ER. In step 4, the signal sequence is cleaved by the signal peptidase (not shown), the TRAM protein disengages, and the nascent chain adopts a transmembrane structure.

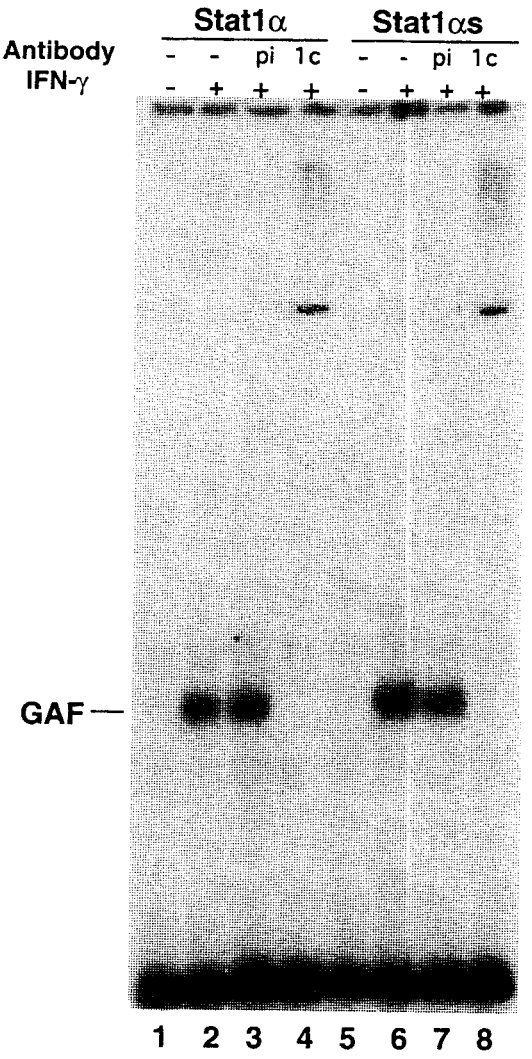
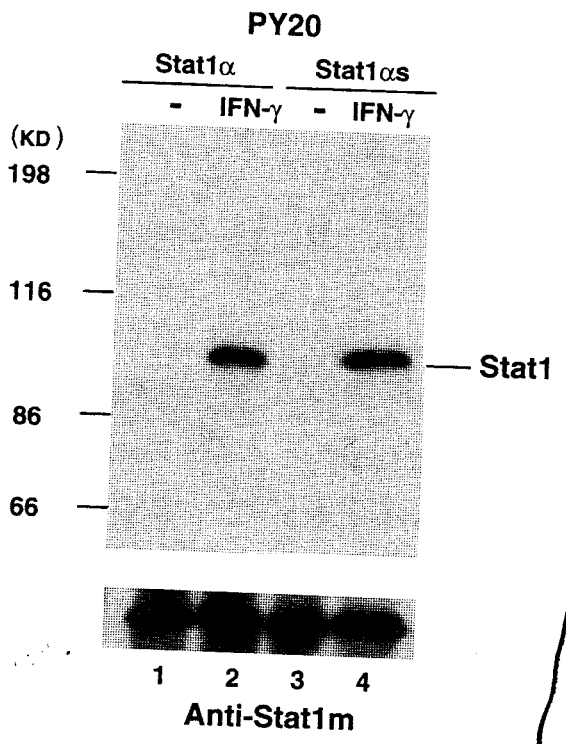


Fig 2

(A) Gel mobility shift analysis was performed with ^{32}P-labeled IRF-1 GAS probe. Nuclear extracts were prepared from [redacted] Stat1α cells (lanes 1–4) and [redacted] Stat1αs (the Ser-727→Ala-727 mutant, lanes 5–8) cells untreated (lanes 1 and 5) or treated with IFN-γ (lanes 2–4 and 6–8). Preimmune serum (pi, lanes 3 and 7) or anti-Stat1C serum (1C, lanes 4 and 8) was added in the reaction.

Fig 3

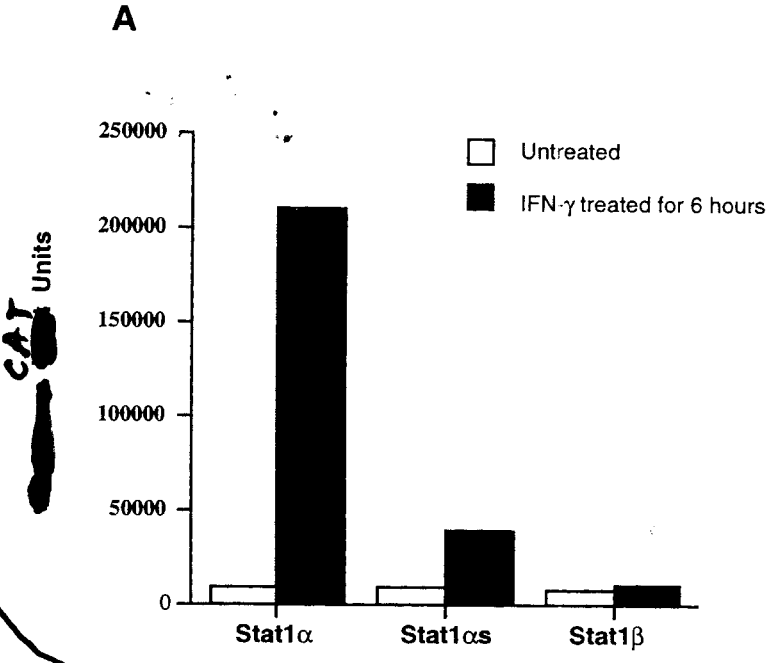


(C) Protein immunoblot of anti-Stat1C precipitates with anti-phosphotyrosine antibody. Whole-cell lysate from [redacted] Stat1α cells (lanes 1 and 2) or [redacted] Stat1αs cells (lanes 3 and 4) either treated with IFN-γ (lanes 2 and 4) or untreated (lanes 1 and 3) were precipitated with anti-Stat1C serum and probed with anti-phosphotyrosine antibody (top). The blot was then stripped and reprobed with anti-Stat1 middle serum (anti-Stat1m, bottom).

4

Transcriptional Activation Analysis of Stat1αs

(A) [redacted] assay. Transfection into [redacted] cells of a reporter gene ([redacted]) plus either Stat1α, Stat1αs, or Stat1β expression constructs for 16 hr was followed by a 6 hr IFN-γ treatment; extracts were prepared, and [redacted] assays were carried out. [redacted] units are plotted on the Y axis (open bars, untreated cells; closed bars, IFN-γ-treated cells).



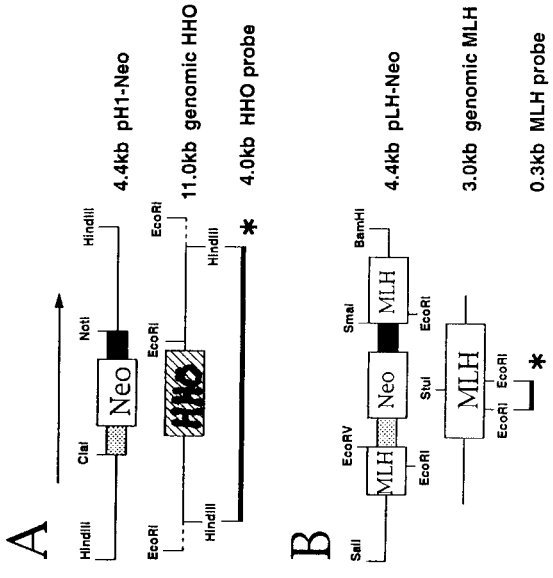


Figure 1. Disruption Constructs and [redacted] Genomic Maps of the *HHO* and *MLH* Loci

(A) Map of the [redacted] *HHO* gene and the *HHO*-replacement insert. A 4.4 kb HindIII fragment of pH1-Neo is shown. A 1.4 kb drug resistance marker was inserted between the ClaI and NotI sites introduced by PCR. The stippled box is the *HHF1* promoter, followed by the *neo* gene and the *BTU2* terminator (shown as a closed box). This insert does not contain an EcoRI site. The [redacted] genomic *HHO* gene is shown as a hatched box in a 11.0 kb EcoRI fragment. One EcoRI site is adjacent to the end of the H1 coding region. A 4.0 kb HindIII fragment used as an *HHO* probe in the Southern and Northern blot analyses is shown as a bold line with an asterisk at its end.

(B) Map of the [redacted] *MLH* gene and the *MLH* disruption insert. A 4.4 kb SalI–BamHI fragment of pLH-Neo is shown. The *neo* marker with EcoRV and SmaI blunt ends was inserted into a StuI site in the coding region of the *MLH* gene. The [redacted] genomic *MLH* gene is shown as a 3 kb fragment containing the MLH coding region (shown as an open box). The *MLH* probe is a 0.3 kb EcoRI fragment from the *MLH* coding region (shown as a bold line with an asterisk at its end). All genes are transcribed in the direction indicated by the arrow.

Figures pg 2

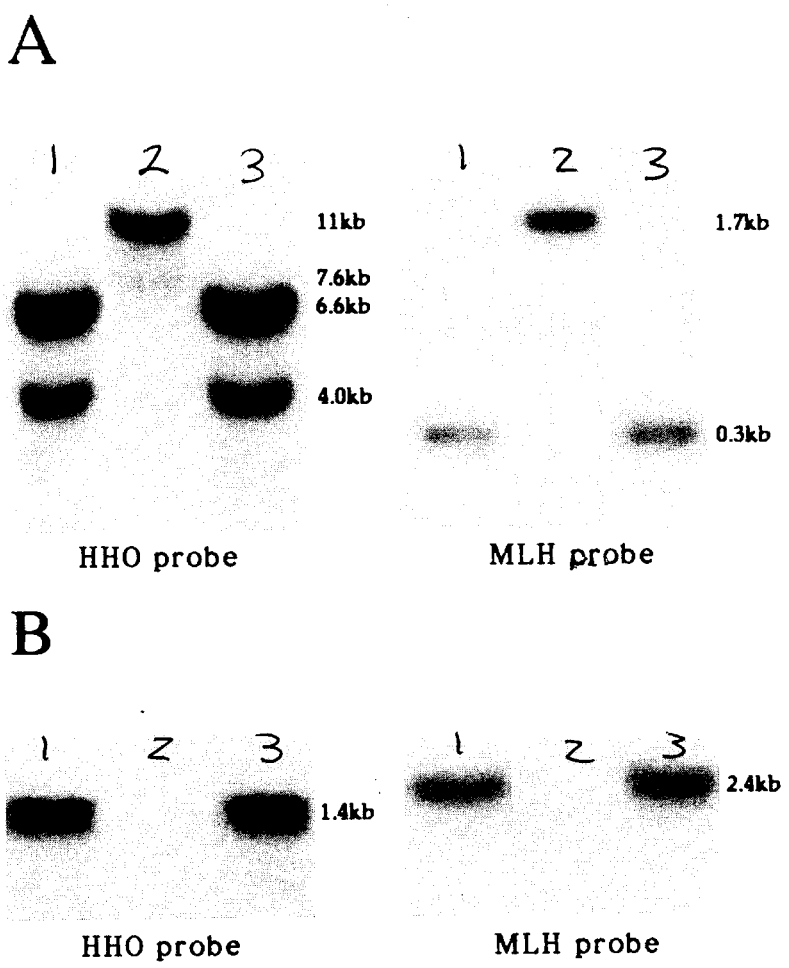
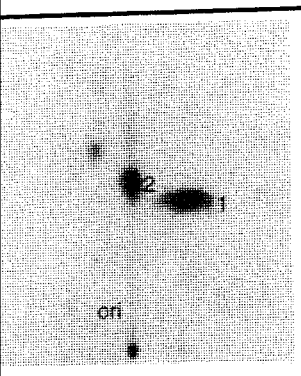


Figure 2. Southern and Northern Blot Analyses of [redacted]
Strains [redacted]

(A) Southern blot analysis, [redacted] Total cell DNA (15 µg) was digested with EcoRI. Using the 4.0 kb *HHO* probe, two strong bands (4.0 kb and 6.6 kb) are detected [redacted] as well as [redacted] an 11.0 kb band [redacted] Using the 0.3 kb *MLH* probe, a 0.3 kb band can be seen [redacted] and [redacted] a 1.7 kb band [redacted]

(B) Northern blot analysis of total cell RNA [redacted] Total RNA (15 µg) was analyzed in each lane. Using the *HHO* probe, a 1.4 kb H1 message can be seen [redacted] Using the *MLH* probe, a 2.4 kb MicLH message band is seen [redacted]

Figures Pg 3



equally phosphorylated on tyrosine, as tested by anti-phosphotyrosine antibody reaction with Stat1 immunoprecipitates separated on polyacrylamide gels (Figures 4B and 4C).

Requirement for Ser-727 in Stat1α Transcriptional Induction

Having demonstrated that serine phosphorylation of residue 727 in Stat1 occurs in vivo and that mutant protein lacking this residue could be phosphorylated on tyrosine and bind DNA, we tested for the effect on IFN-γ-induced transcription. Two different experiments indicated that the Ser-727 was required for maximal IFN-γ-induced transcription. First, U3A cells were transfected with either wild-

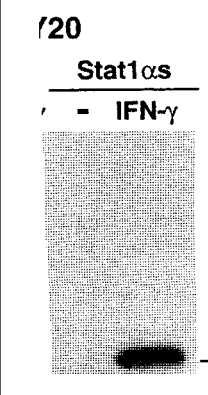


Figure 4. DNA Binding and Tyrosine Phosphorylation of Ser-727→Ala-727 Mutant of Stat1α in U3A-Complemented Cells

(A) Gel mobility shift analysis was performed with ^{32}P-labeled IRF-1 GAS probe. Nuclear extracts were prepared from U3A Stat1α cells (lanes 1–4) and U3A Stat1αs (the Ser-727→Ala-727 mutant, lanes 5–8) cells untreated (lanes 1 and 5) or treated with IFN-γ (lanes 2–4 and 6–8). Preimmune serum (pi, lanes 3 and 7) or anti-Stat1C serum (1C, lanes 4 and 8) was added in the reaction.

(B) Western blot analysis. Whole-cell lysate from U3A cells (lane 1), U3A cells comple-