7

Molecular Biology 2nd Exam - spring 199

ALL EXAMS ARE DUE AT 8:30 am ON MONDAY APRIL 7.

There is no time limit on this test. You may find it easier to take this test over several days, though if you are confident in your molecular skills, you could wait until Sunday night. However, I predict it will take many of you a bit longer to think of all the answers (just some friendly advice). You are not allowed to use your notes, any books or journals, nor are you allowed to discuss the test with anyone until all exams are turned in at 9:30 am on Monday, April 7. You may use a calculator and/or a ruler and graph paper. The answers to the questions must be typed, though you may want to supplement your text with hand drawn figures (write neatly for any labels in your figures).

-3 pts if you do not follow this direction.
Please do not write 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):

Write out the full pledge and sign:

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

10 pts.

1) As you can see from this first abstract (figure 1), a lot of scientists find it difficult to write in plain English.

Please summarize this abstract in your own words using as little jargon as possible. Your summary should NOT be longer than the original summary.

10 pts.

2) Figure 2 shows some DNA footprint analysis. CC+20 is a particular sequence of DNA. CRP is a protein, as is RNA polymerase. Notice that the top line of + or - is indicating the presence or absence of CRP; the bottom line indicates the presence or absence of RNA polymerase. The numbers to the right of the data are intended to be used as a yard stick for your convenience.

Please interpret these data as completely as possible.

10 pts.

3) Figure 3 is examining the role of different alleles of p53 in tumor formation. Note that Giesma stain lables DNA and thus what you see are dark spots of foci; these spots are evidence of rapid cellular proliferation.

Please interpret these data. Note that p53dl has no functional p53.

10 pts.

4) Figure four shows a band shift assay in which the promoter DNA is radioactive (or "hot") and every lane has purified transcription factor E2F added to it. A "-" indicates that there is **no** addition of identical promoter DNA sequence that is non-radioactively labeled (no "cold" DNA added), while "+" indicates that there was addition of identical promoter DNA sequence that is non-radioactively labeled ("cold" DNA added). The molecular weight of E2F plus promoter is indicated by the arrow. The labels at the top indicate the source of additional cell extracts. For example, L means that the cell line "L" was homogenized and then these proteins were added to the tube for the pair of lanes (- and +) before the band shift gel was run.

a) Explain the data you see.

b) What is the purpose of the "+" lanes?

10 pts.

5) Here is another band shift assay (figure 5) . This time we will compare three different binding proteins (HMG-1, MATH20, and MATH10) and two different promoters (SAR and SV40). Make the assumption that all these lanes were analyzed on a single get so there is no variation due to differences in different gels. Note that the first lanes in panels A and C are negative signs and not the number one; these lanes had no protein added to the assay.

Interpret these data

10 pts.

6) Figure 6 is a set of results from CAT assays. NF-kB, IRF-1, and ATF2/cJun are three transcription factors. The two different reporter plasmids vary in their promoter sequences such that the DNA helix has been added to by half a rotation (panel B) compared to the promoter in panel A. As a result, all the sequences on one side of the double helix that were pointing up (panel A) are now pointing down (panel B).

What can you conclude from these data?

10 pts.

7) Figure 7C shows hydropathy plots for two related molecules (FRL1 and FRL2). Assuming that their structures are well conserved and that differences in amino acid sequence have no major impact on overall structure, what can you deduce about the structures/topology of FRL1 and FRL2?

10 pts.

8) Given the data in figure 8A - 8C, what can you conclude about FRL1 and FRL2 expression?

10 pts.

9) Determine the chromosomal location of XRCC. (It must be nice to have the data for an entire figure donated to you by another researcher!)

10 pts.

10) And the final question...

Figure 10 shows a series of immunofluorescence micrographs using NIH3T3 cells (which is generic cell line that many researches grow in culture) that have been infected/transfected as described in the legend. HA stands for Hemagglutinin which is used as an epitope tag similar to the c-myc tag. The anti-p19 antibody was generated by injecting a mouse with a (cognate) peptide from the full-length p19. Panels D and E have the exact same NIH 3T3 cells grown in the presence of a modified form of dUTP (called BrdU in panel E) which can be incorporated into growing DNA strands in place of dTTP, and can be detected by a monoclonal antibody.

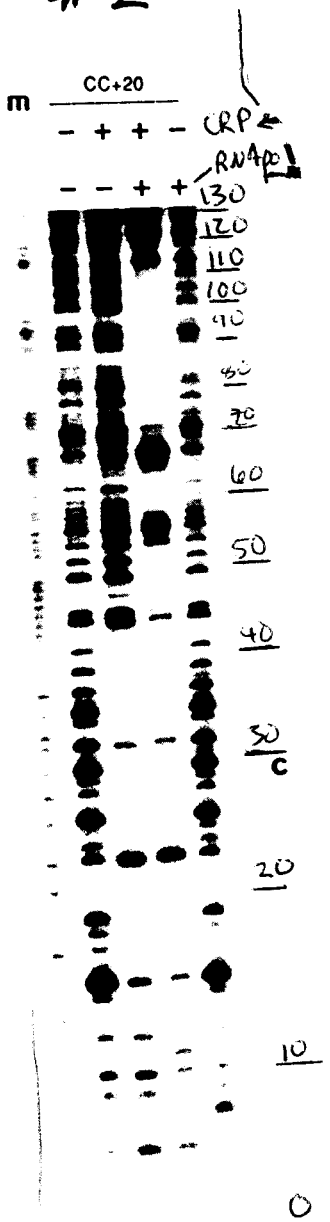
Please interpret panels A, B and C, and then tell me the conclusion from panels D and E.

#1

Summary

A proposed mechanism for sorting secretory proteins into granules for release via the regulated secretory pathway in endocrine-neuroendocrine cells involves binding the proteins to a sorting receptor at the *trans*-Golgi network, followed by budding and granule formation. We have identified such a sorting receptor as membrane-associated carboxypeptidase E (CPE) in pituitary Golgi-enriched and secretory granule membranes. CPE specifically bound regulated secretory pathway proteins, including prohormones, but not constitutively secreted proteins. We show that in the *Cpe*^{fat} mutant mouse lacking CPE, the pituitary prohormone, pro-opiomelanocortin, was missorted to the constitutive pathway and secreted in an unregulated manner. Thus, obliteration of CPE, the sorting receptor, leads to multiple endocrine disorders in these genetically defective mice, including hyperproinsulinemia and infertility.

#2



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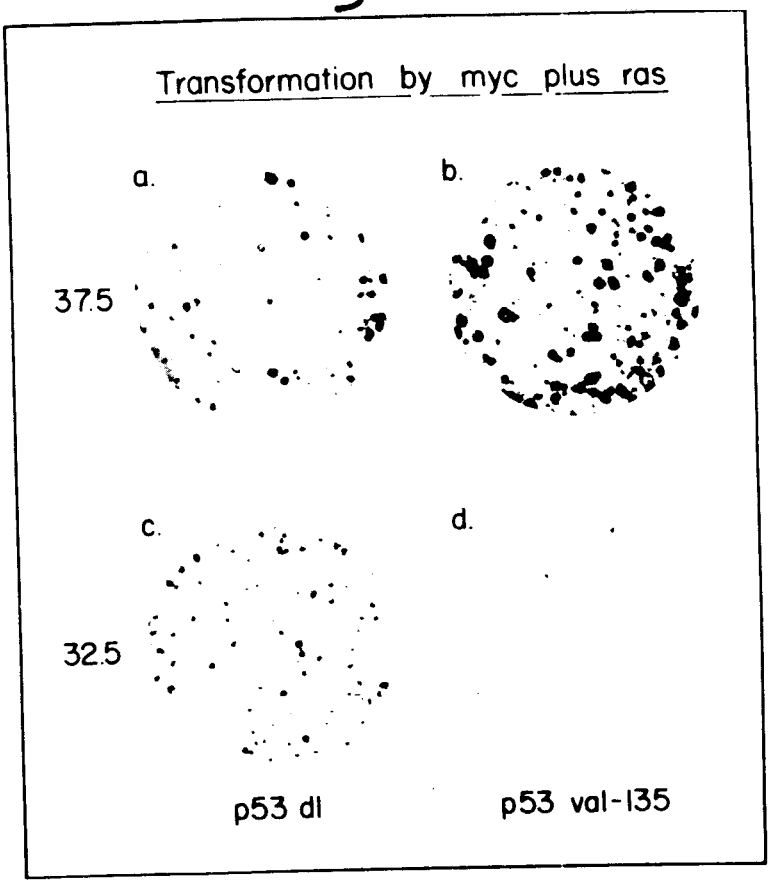


Figure 3. Effect of p53val135 on Transformation by *myc* + *ras* at Different Temperatures

A combination of plasmids encoding *myc* (1 μg) and *ras* (1.5 μg) was cotransfected into REFs together with a plasmid encoding p53val135 (1.5 μg) or with a control plasmid (pLTRp53dl, 1.5 μg). Cells were maintained at 37.5°C for 9 days or at 32.5°C for 13 days, after which cultures were stained with Giemsa stain.

#4

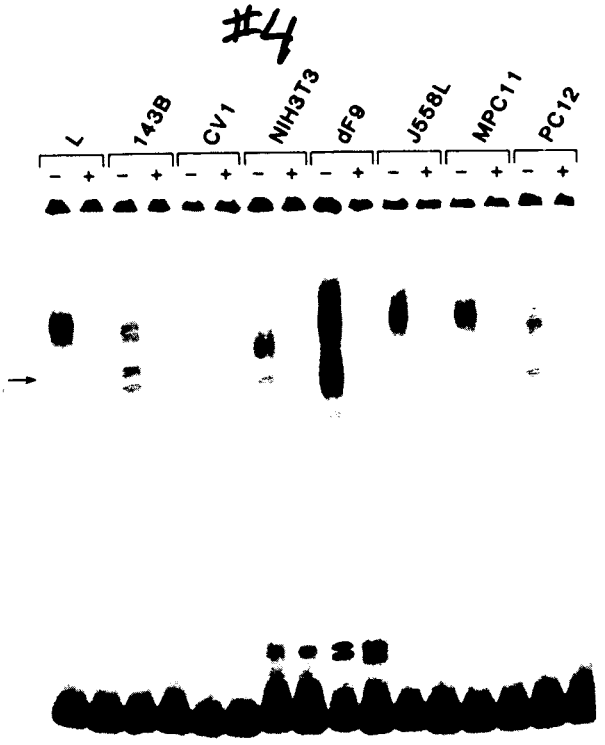


Figure 4 Heterogeneous E2F Binding Activity in Extracts of Various Cell Lines

#5

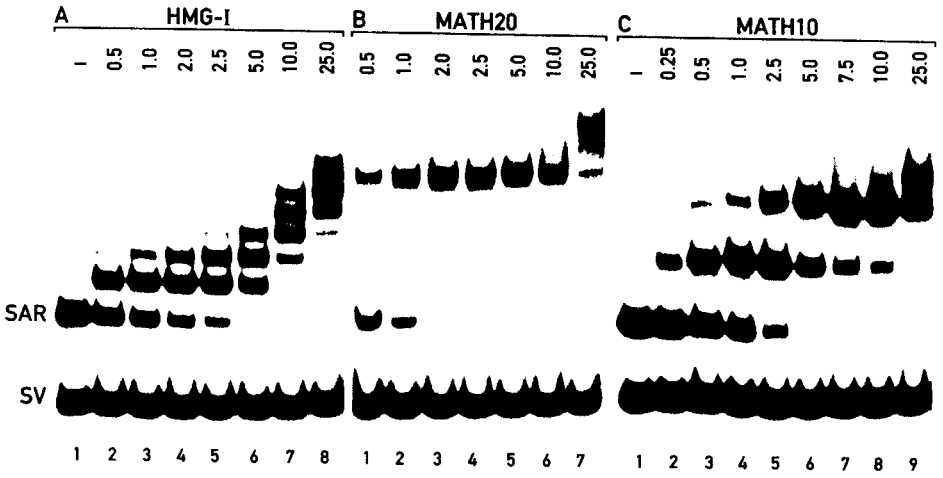


Figure 5. MATH Proteins Bind SAR DNA with Great Specificity

The 310 bp SAR subfragment (SAR), derived from the Drosophila histone SAR (Mirkovitch et al., 1984), and the 223 bp non-SAR fragment (SV) containing the SV40 promoter region (Zhao et al., 1993) were end-labeled and analyzed by band shift with an increasing amount of HMG-I (A), MATH20 (B), and MATH10 (C) in nanograms, as indicated.

#6

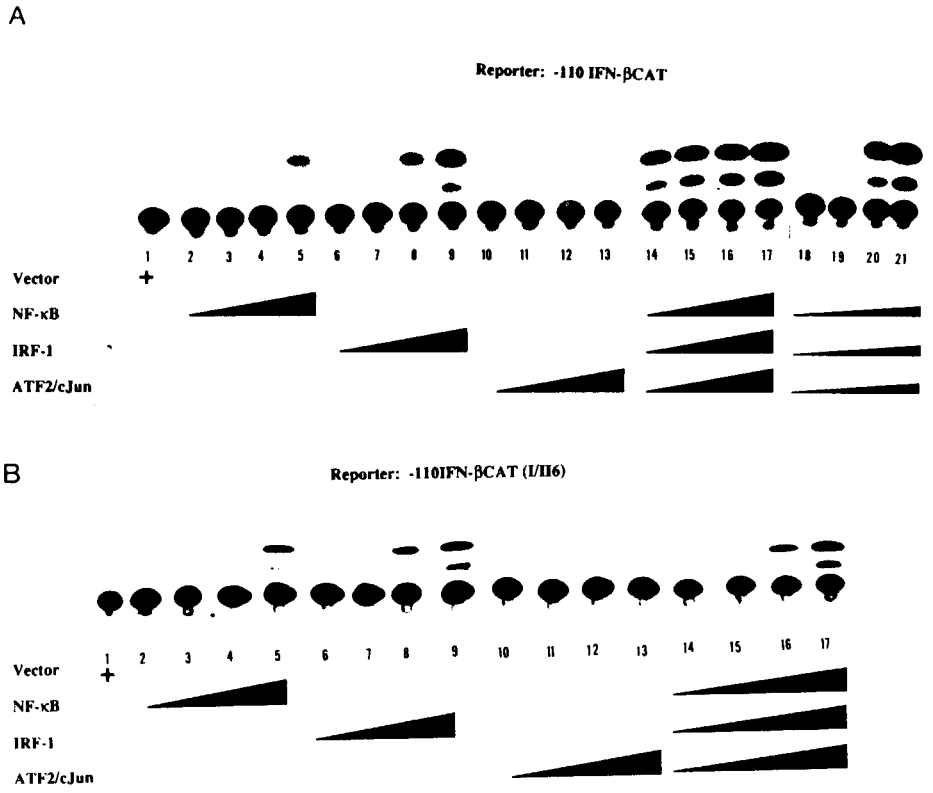
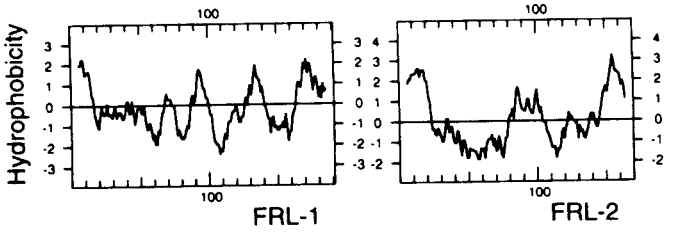


Figure 6 Synergistic Activation of the IFNβ Gene Enhancer in Cultured Mammalian Cells

Mouse embryonal P19 cells were cotransfected with the wild-type IFNβ reporter plasmid (A) or the helical mutated reporter (B), along with increasing amounts of expression vectors directing the synthesis of NF-κB ATF-2 plus c-Jun, and IRF-1. Lane 1, 9 μg of empty expression vector. Lanes 2–5, increasing amounts of an equimolar mixture of NF-κB expression plasmids (100 ng, 300 ng, 1 μg, and 3 μg). Lanes 6–9, increasing amounts of *IRF-1* expression plasmid (100 ng, 300 ng, 1 μg, and 3 μg). Lanes 10–13, increasing amounts of an equimolar mixture of *ATF-2* and *c-jun* expression plasmids (100 ng, 300 ng, 1 μg, and 3 μg). Lanes 14–17, increasing amounts (100 ng, 300 ng, 1 μg, and 3 μg) of all the expression vectors. Lanes 18–21, increasing amounts (3 ng, 10 ng, 30 ng, and 100 ng) of all the expression vectors (A).

#7

7C



#8

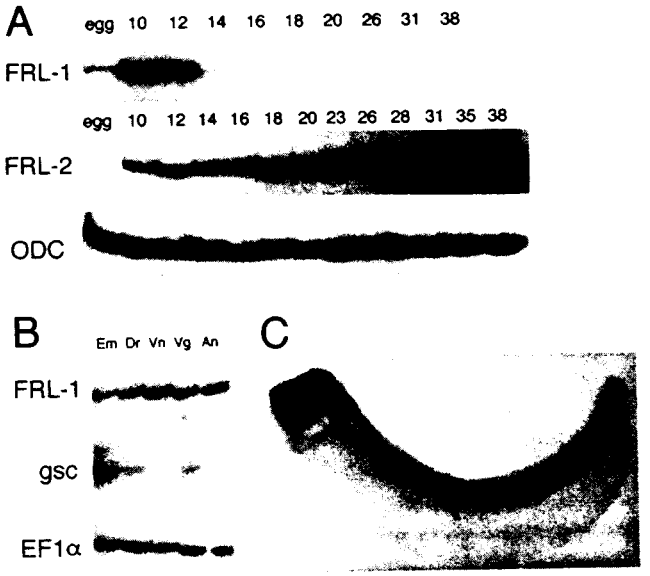


Figure 8. The Temporal and Spatial Expression of *FRL1* and *FRL2* Genes during Embryogenesis

(A) Temporal expression. RNA was isolated from unfertilized eggs and the indicated stages of embryos. The expression of the *FRL1* and *FRL2* genes was assayed by RT-PCR. Ornithine decarboxylase (ODC) is present as an internal control.

(B) Spatial expression. Stage 10 embryos were dissected into the dorsal half (Dr) and the ventral half (Vn), or into the vegetal half including the marginal zone (Vg) and the animal half (An), and RNA was isolated from these tissues or from stage 10 embryos (Em). The expression of the FRL1 gene was assayed by RT-PCR. *goosecoid* (*gsc*) is known to be expressed in the dorsal marginal zone (Cho et al., 1991). EF1α is a ubquitously expressed control (Krieg et al., 1989).

(C) Whole-mount in situ hybridization in the tailbud stage embryo of *FRL2* RNA. Purple staining indicates the localization of the antisense *FRL2* probe.

#9

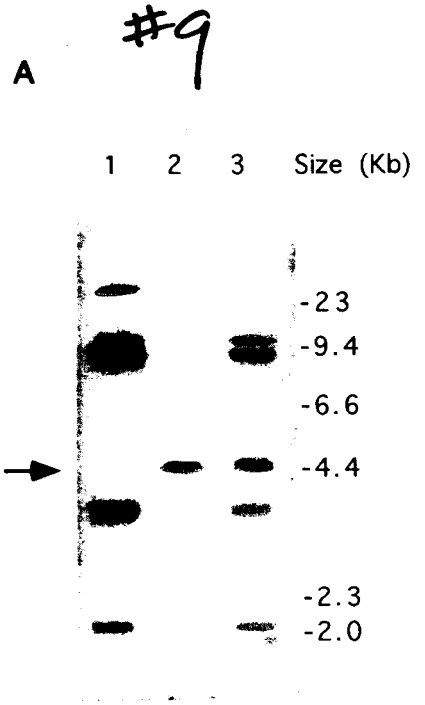


Figure 9 Chromosomal Localization of Human *XRCC4* Gene

(A) Genomic DNA from indicated cell lines (Lien et al., 1991) was digested with TaqI and assayed by Southern blotting methods for hybridization to the human *XRCC4* probe. Lane 1, human DNA; lane 2, DNA from a CHO-human hybrid cell line with a single human chromosome 5 missing the region 5q11.2-13.3; lane 3, DNA from a CHO-human hybrid cell line with a normal human chromosome 5 as the sole human component. Arrow points to the endogenous hamster *XRCC4* gene in the CHO-human hybrids. This blot was provided by Dr. L. Kunkel.

#10

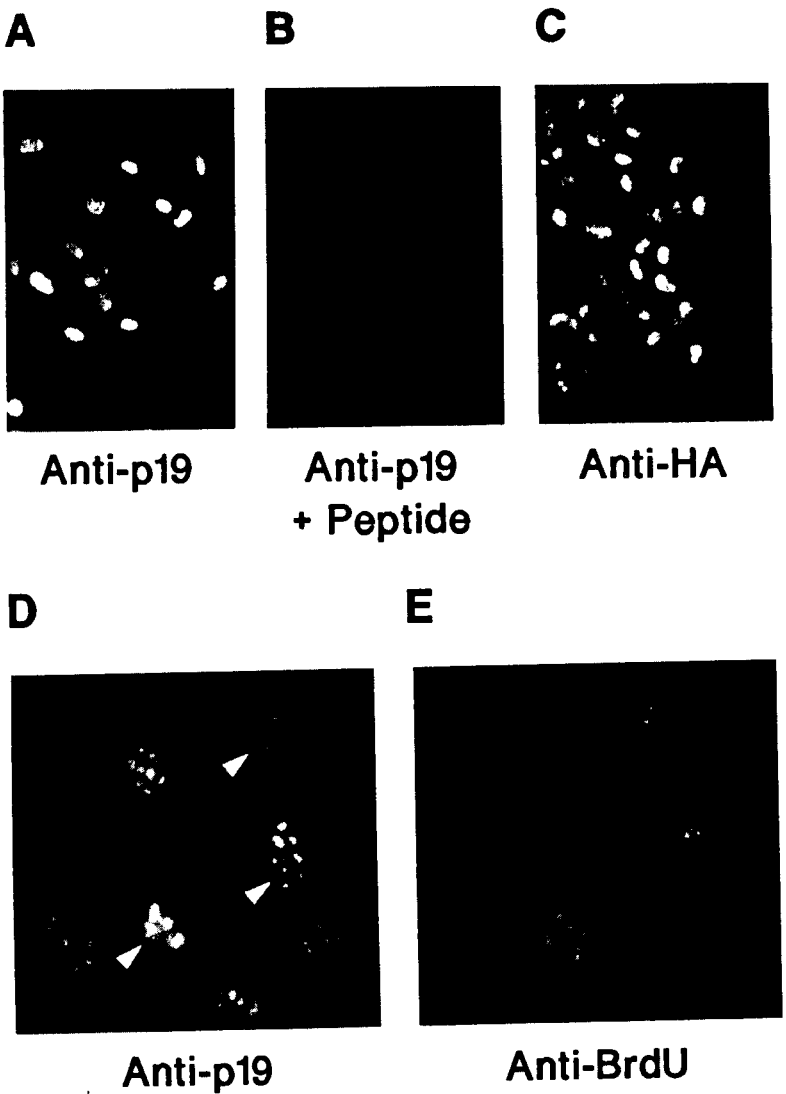


Figure 10 [redacted] Localization of p19^{ARF}

Cytospin preparations of NIH 3T3 cells infected for 48 hr with a vector encoding HA-tagged p19^{ARF} were fixed and stained with antiserum to p19*ARF* (A), anti-p19^{ARF} plus cognate peptide (B), or anti-HA serum (C)