2000 Molecular Biology Exam #2 – Using Molecular 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 11:30 am on Monday March 27, 200. **EXAMS ARE DUE AT 11:30 AM ON MONDAY, March 27**. 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.

-3pts 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)?

You have called the 1-800 number for a new game show called "Who wants to be a Molecular Biologist?!" The host, Reagent Philament has created some screening questions that will separate the "wanna' be's" from the hard core molecular biologists.
Press 1 to continue.

Many of the figures are on the last pages of this test, while others are embedded in the questions. Good luck and remember in this game you are not allowed to use any life lines.

10 pts.

1. For $200. Figure 1 shows some results that are very exciting. Please interpret the results for panel B as fully as you can. If you cannot see what you think you should on your paper version, you can find an electronic copy of this figure here, but do not print it.
<www.bio.davidson.edu/Biology/Courses/Molbio/exams/2000/exam2_2000.html>

10 pts.

2. For $400. This question has some figures that are **not** necessary to answer the question, but help you understand the importance of this work. Go to the same URL to see the four additional figures.

a) Read the abstract.

b) Angiogenesis is the biological process of forming new blood vessels. Remember that blood vessels are lined with endothelial cells.

c) Design a series of procedures that would allow you to purify angiostatin for the first time. Up to this point (for the sake of the test) no one has any idea what molecule(s) is/are inhibiting tumor growth. You job is to find that/those molecules. Your only hint is that the molecule(s) does not work if it is boiled first. I will tell you that this group started with 20 liters of mouse urine, so you know it is not found in high concentrations.

15 pts.

3. For $800. A new *Drosophila* protein has been identified via its cDNA. The cDNA was generated from flight muscles. This fly cDNA was used to probe a blot using muscle tissues from three species. On the gel was loaded: 1 μg of Rat mRNA, 2 μg of Chicken mRNA, and 5 μg of *Drosophila* mRNA. See figure 2.

An electronic version for this is also available if you want to see it (same URL as in question 1)

a) Interpret figure 2 as fully as you can.

b) What control (if any) has been run on this blot?

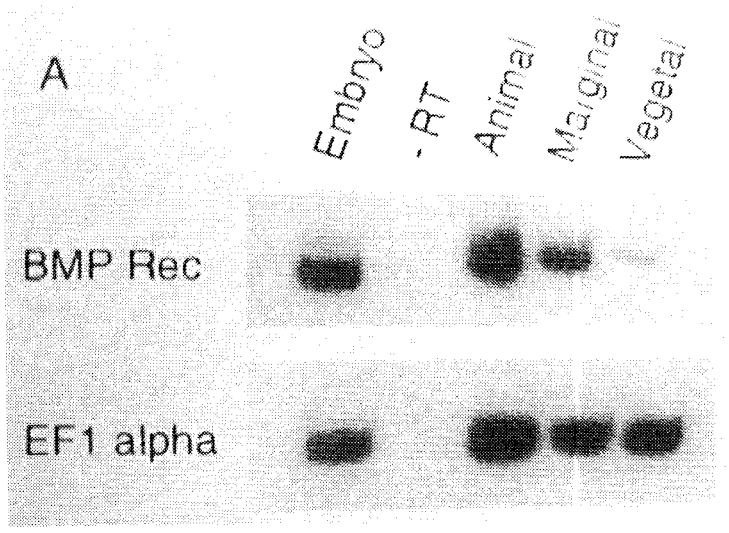
c) What do you think the purpose was for figure 2?

15 pts.

4. For $1600.

The figure here (no.3) ----------→ shows the results of an experiment using mRNA and RT-PCR. RT-PCR uses reverse transcriptase and then PCR to produce bands on a gel. The frog embryos were dissected at stage 8 and broken into three parts, or used whole (embryo lane). EF1 α is an elongation factor used during translation.

a) What is the purpose of using EF1α?



b) What is the purpose of leaving out RT in the lane marked "-RT" ?

c) What is the bottom line for this figure?

10 pts.

5. For $8000. Figure 4 shows a summary for a lot of work. The question being asked in this experiment is "Which amino acids of mouse BRG1 are necessary to bind to mouse retinoblastoma (RB) protein [which is a powerful tumor suppressor]?"
The figure shows the amino acid numbers along the top and boxes indicating which portions of the full length protein (which is 283 amino acids long) are being expressed in each experiment. The black boxes (first 3 constructs from the top) showed full binding; the next 6 constructs are shaded gray and indicate partial binding to RB; the 5 white boxes indicate which constructs failed to bind to RB at all.
a) Does BRG1 have one linear sequence that binds to RB? Explain your answer.
b) Which amino acids play a direct role in binding to RB?

20 pts.

6. For $16,000. The abstract by Yin *et al.* (1994) show why *Drosophila* is a great model system for neurobiology. Cycloheximide is a drug that blocks translation of mRNA into protein. CREB is a transcription factor that must bind to cAMP before it can be functional. A dominant negative protein means that it is a dominant protein that blocks the wild-type proteins from functioning properly. ARM can be thought of as short-term memory.
a) How can a dominant negative allele work?
b) What can you conclude about LTM since it is cycloheximide sensitive?
c) What can you conclude about LTM since it was completely blocked when the dominant negative transgene of CREB was expressed?
d) From these experiments, what can you conclude about the differences between ARM and LTM?

10 pts.

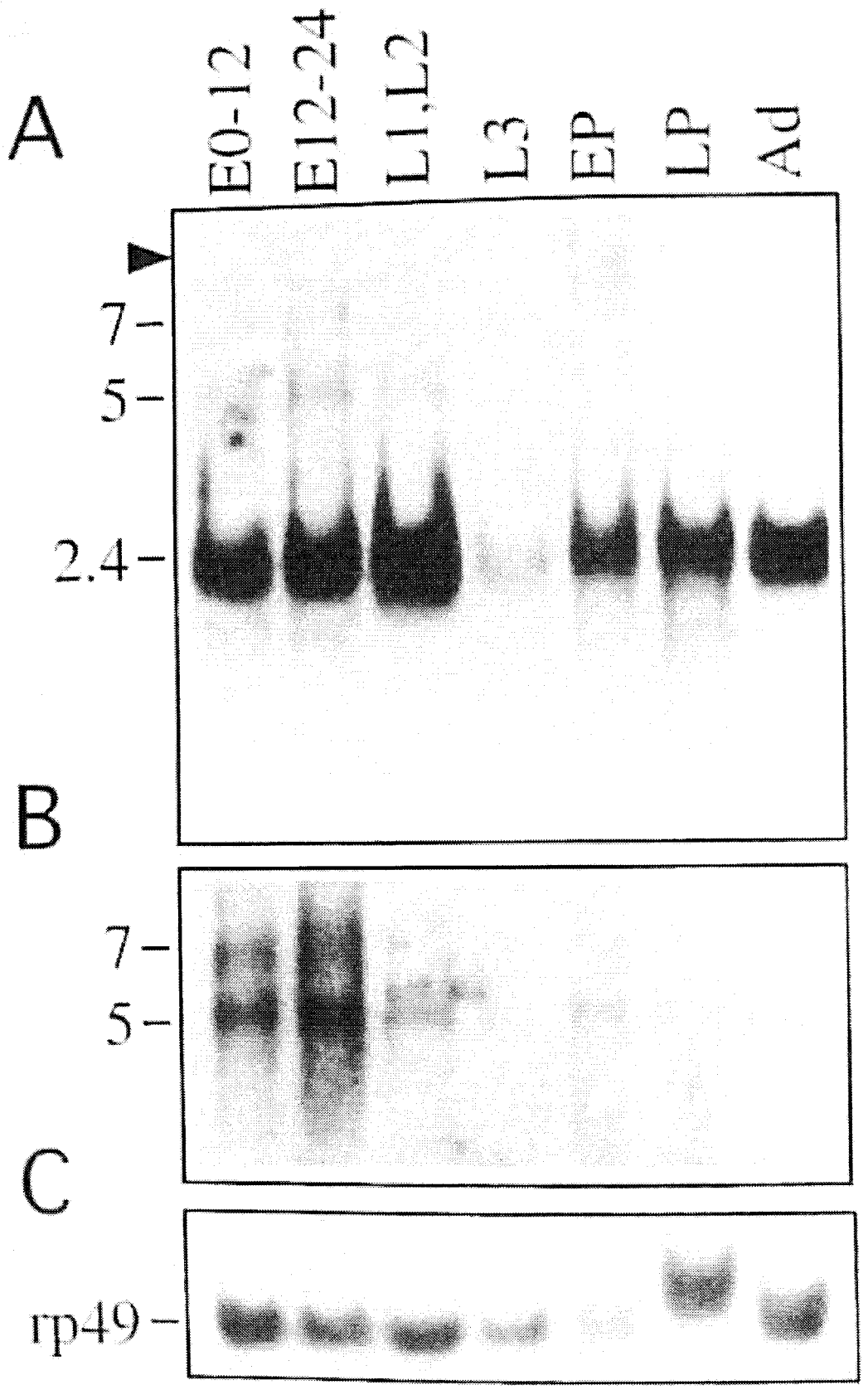
7. For $32,000. One paper was entitled "The cytoplasmic domain of the X receptor is **Sufficient** to couple the receptor to its signal transduction pathway".
a) What type of experiment did the authors do to say it was sufficient?
b) What type of experiment would the authors need to do to say it was necessary?

10 pts.

8. For $64,000. The figure on the next page is asociated with the figure legend # 5 at the end of this test. Are there any statements made in the figure legend that are not supported by the data? You MUST explain your answer(s) to receive full credit.

Is that your final answer?

Sadly, the sponsors of the contest must confess that they were unable to raise enough money to continue this fun game. However, stay tuned for the newest idea, "Who wants to marry a Molecular Biologist?!" (the correct answer being "who wouldn't ?")


A
E0-12
E12-24
L1,L2
L3
EP
LP
Ad
7
5
2.4
B
7
5
C
rp49

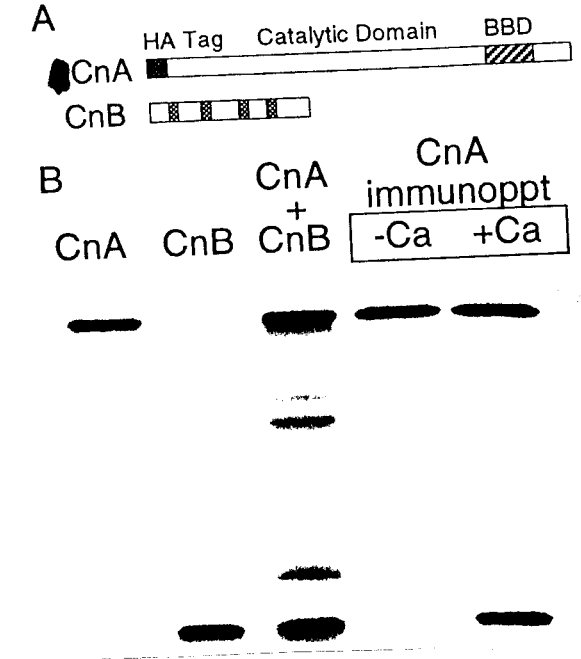


Figure 1. Expression and Functional Analysis of Calcineurin Subunits

Human cDNAs encoding the catalytic (CnA) and regulatory (CnB) subunits were cloned into pSP73 and expressed

(A) Structure of the CnA subunit modified with an N-terminal epitope tag (hemagglutinin, HA) recognized by the 12CA5 monoclonal antibody. The CnB subunit has four calcium-binding domains and was expressed in its native form. BBD, CnB-binding domain.

(B) Lane 1, CnA expression alone; lane 2, CnB alone; lane 3, cotranslation of CnA and CnB; lane 4, immunoprecipitation (immunoppt) of CnA from with both CnA and CnB at low calcium (Ca) concentration; and lane 5, immunoprecipitation of CnA from with CnA and CnB in the presence of 1 mM $CaCl_2$.

cells

Fig 1

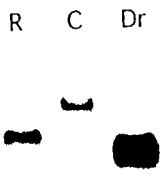


Fig 2

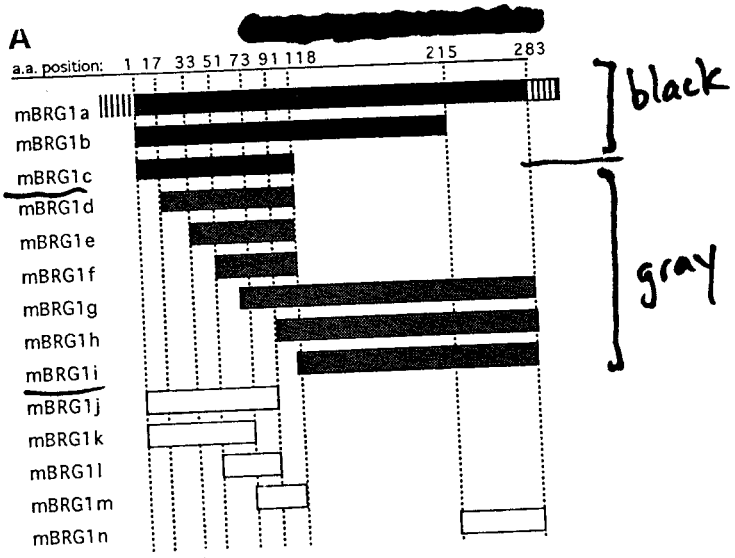


Fig 4

Fig 5 legend

Figure 5 Developmental Northern Blot Analysis of *eas* cDNAs

The stages represented are 0–12 hr (0–12E) and 12–24 hr (12–24E) embryos, first and second larval instars (L1, L2), third instar (L3), early (EP) and late (LP) pupae, and adults (Ad).

(A) Probed with clone 12, corresponding to the common region of the isolated cDNAs. At least three transcripts are detected, indicated by ticks, with sizes in kilobases. The arrowhead indicates an artifactual band that we often observed with different probes. The most prominent transcript (2.4 kb), expressed in all stages, corresponds approximately in size to clone 12. A similar hybridization pattern was obtained with genomic probes from the *eas* locus.

(B) A probe specific for clone 5 (a 1 kb BsmI fragment from exon I) hybridizes to an ~5 kb transcript detectable in all stages except third instar. This probe also hybridizes to a larger (~7 kb) transcript detectable only in embryonic stages.

(C) Ribosomal protein 49 probe (rp49) (O'Connell and Rosbash, 1984), included as a loading control.

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Summary

Consolidated memory after olfactory learning in Drosophila consists of two components, a cycloheximide-sensitive, long-term memory (LTM) and a cycloheximide-insensitive, anesthesia-resistant memory (ARM). Using an inducible transgene that expresses a dominant negative member of the fly CREB family, LTM was specifically and completely blocked only after induction, while ARM and learning were unaffected.

Abstract by Yin et al, 1994

Summary

The phenomenon of inhibition of tumor growth by tumor mass has been repeatedly studied, but without elucidation of a satisfactory mechanism. In our animal model, a primary tumor inhibits its remote metastases. After tumor removal, metastases neovascularize and grow. When the primary tumor is present, metastatic growth is suppressed by a circulating angiogenesis inhibitor. Serum and urine from tumor-bearing mice, but not from controls, specifically inhibit endothelial cell proliferation. The activity copurifies with a 38 kDa plasminogen fragment that we have sequenced and named angiostatin. A corresponding fragment of human plasminogen has similar activity. Systemic administration of angiostatin, but not intact plasminogen, potently blocks neovascularization and growth of metastases. We here show that the inhibition of metastases by a primary mouse tumor is mediated, at least in part, by angiostatin.