Molecular Biology Final Exam - spring 1998
ALL EXAMS ARE DUE AT 5 pm on MAY 8, 1998.

I will be out of town all day May 8 so do not wait until Friday to ask questions.

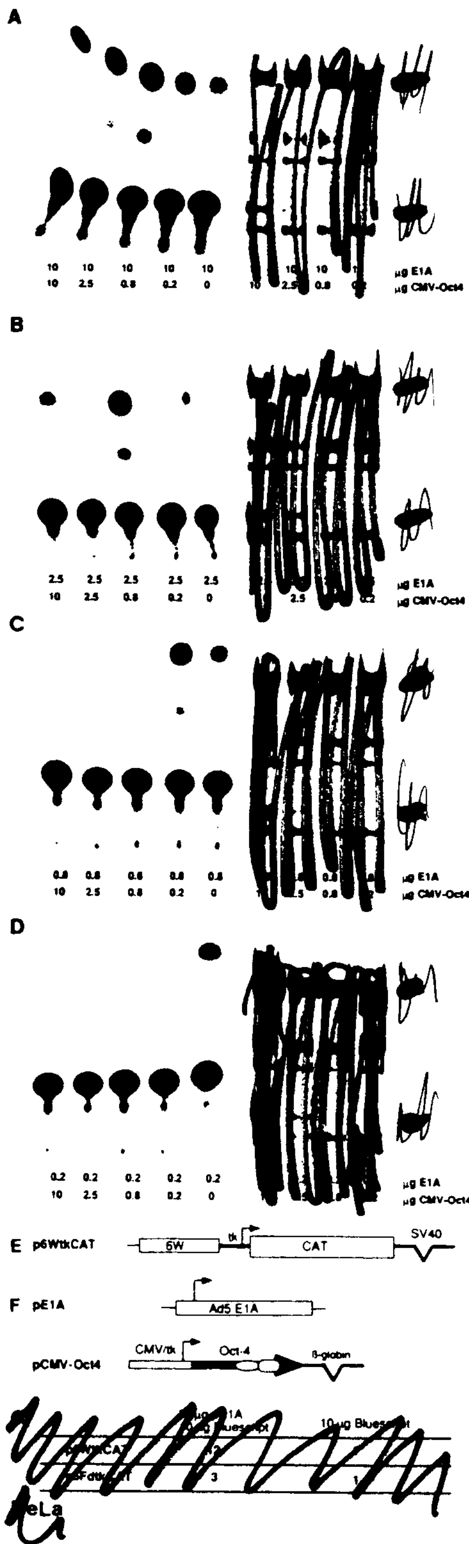
There is no time limit on this test. You may find it easier to take this test over several days, though if you are confident in your molecular skills, you could wait until May 7. However, I predict it will take many of you a bit longer to think of all the answers (just some friendly advice). You are not allowed to use your notes, the WWW, any books or journals, nor are you allowed to discuss the test with anyone until all exams are turned in at **5 pm on MAY 8, 1998**. You may use a calculator, a ruler. 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)?

PAGE A



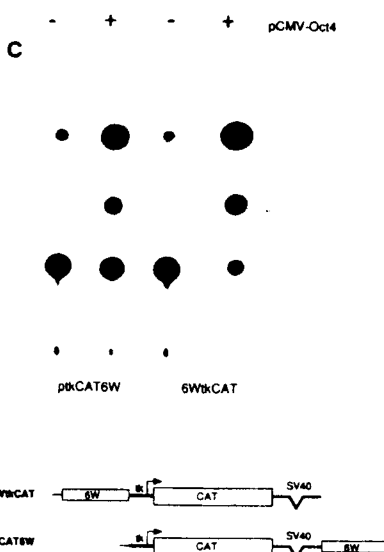


Figure 2.

Line 293 cells were cotransfected with the Oct-4 expression vector (indicated at the top) The extracts were incubated for 1 hr in the CAT assay. In (C) two different reporter plasmids (with 6W either 5′ or 3′ of the CAT gene) were cotransfected with 0.25 µg of Oct-4 expression vector and carrier DNA. Thirty percent of the extract was incubated for 2 hr. In (D) the reporter plasmids

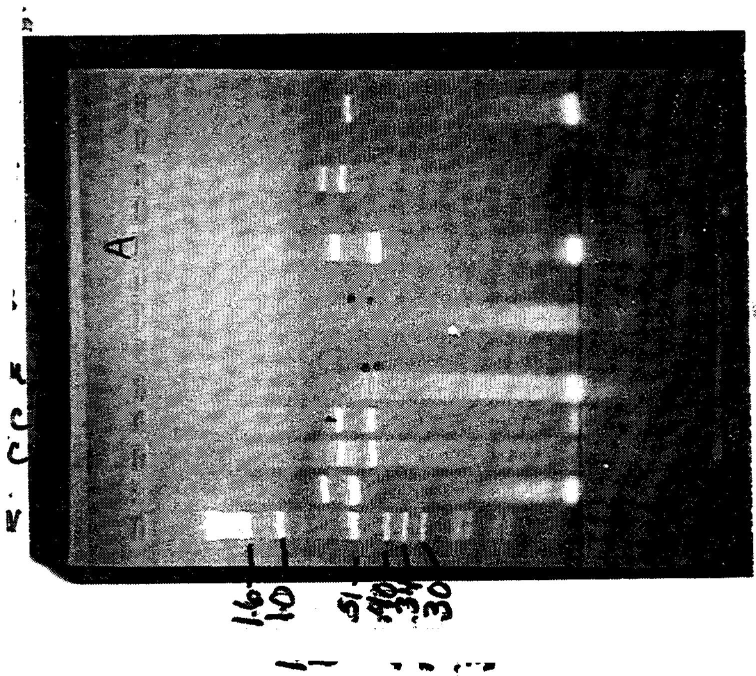


Figure 3.

HeLa cells were cotransfected with different amounts of the Oct-4 and E1A expression vectors (as indicated at the bottom of (A) to (D)) and with p6WtkCAT as a reporter plasmid. Aliquots of the transfected cells were used for the CAT assay (30%; 90 min) The CAT assay is shown at the left and the corresponding EMSA at the right of each panel.

In (E) the reporter plasmid and in (F) the expression vectors used are schematically outlined.

PAGE B

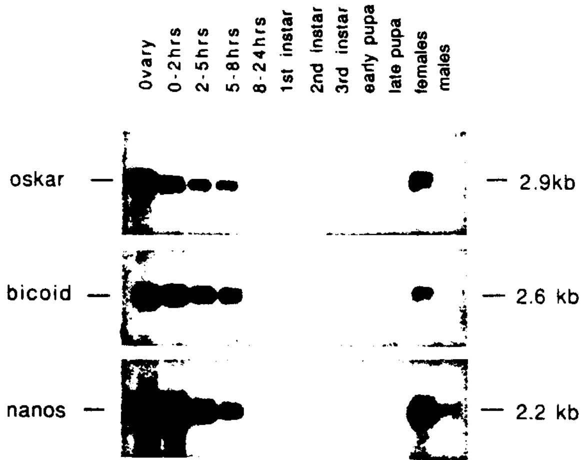


Figure 2. Profile of *osk* RNA Expression during Development

Northern blot of poly(A)$^+$ RNA from all stages of Drosophila development, probed with the 2.3 kb EcoRI fragment.

The profile of expression of two other maternal transcripts, *bicoid* and *nanos*, are shown below. Hybridization with an actin probe demonstrates that equivalent amounts of RNA were loaded in all the lanes (Wang and Lehmann, 1991).

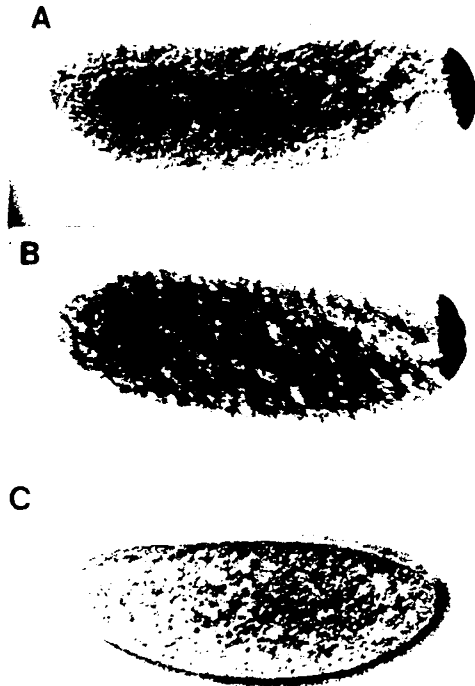


Figure 4. Distribution of *osk* RNA during Embryogenesis

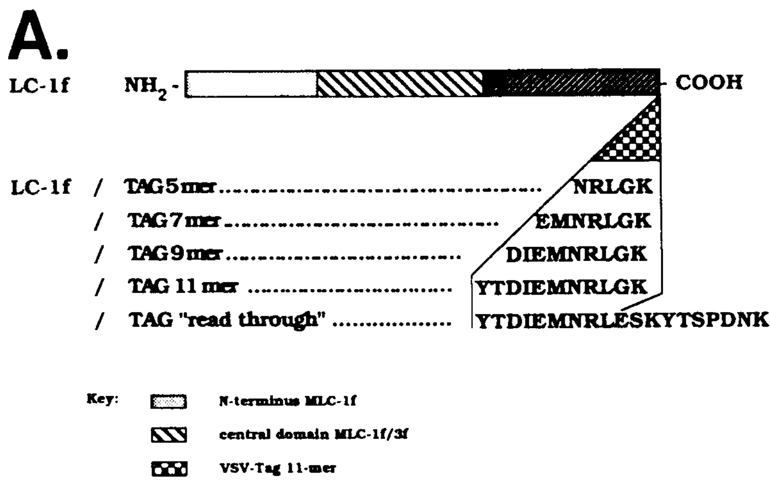
(A) At cleavage stage (stage 2),

(B) At the pole cell stage (stage 3),

(C) By cellular blastoderm stage (stage 5),

Anterior is to the left, and dorsal is up. The stages of embryogenesis are according to Campos-Ortega and Hartenstein (1985).

Fig 3



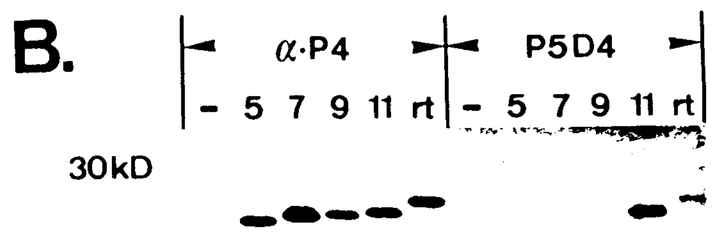


Figure 1. Schematic Representation of the LC-1f Constructs with Different Tag Lengths and Their In Vitro Characterization

(A) The length of the tag (from 5 to 11 amino acids) and its sequence is given. The "read through" mutant has a truncated tag followed by an "out-of-frame" extension of 10 amino acids (see Experimental Procedures). The schematized LC-1f protein is split into three domains, an LC-1-specific N-terminus, an LC-1/LC-3 common region composed of a "divergent" N-terminal half, and a "conserved" C-terminal half.

(B) SDS–PAGE analysis on 15% gels of the immunoprecipitated ^{35}S-labeled proteins derived from the constructs schematized in (A) after in vitro transcription and translation. Anti-tag immunoprecipitations of the untagged LC-1f constructs (lanes marked −) and of LC-1f constructs tagged with different epitope lengths (lanes marked 5, 7, 9, 11, and "rt", respectively) were performed either with polyclonal antibody α-P4 or monoclonal antibody P5D4, respectively.

PAGE C

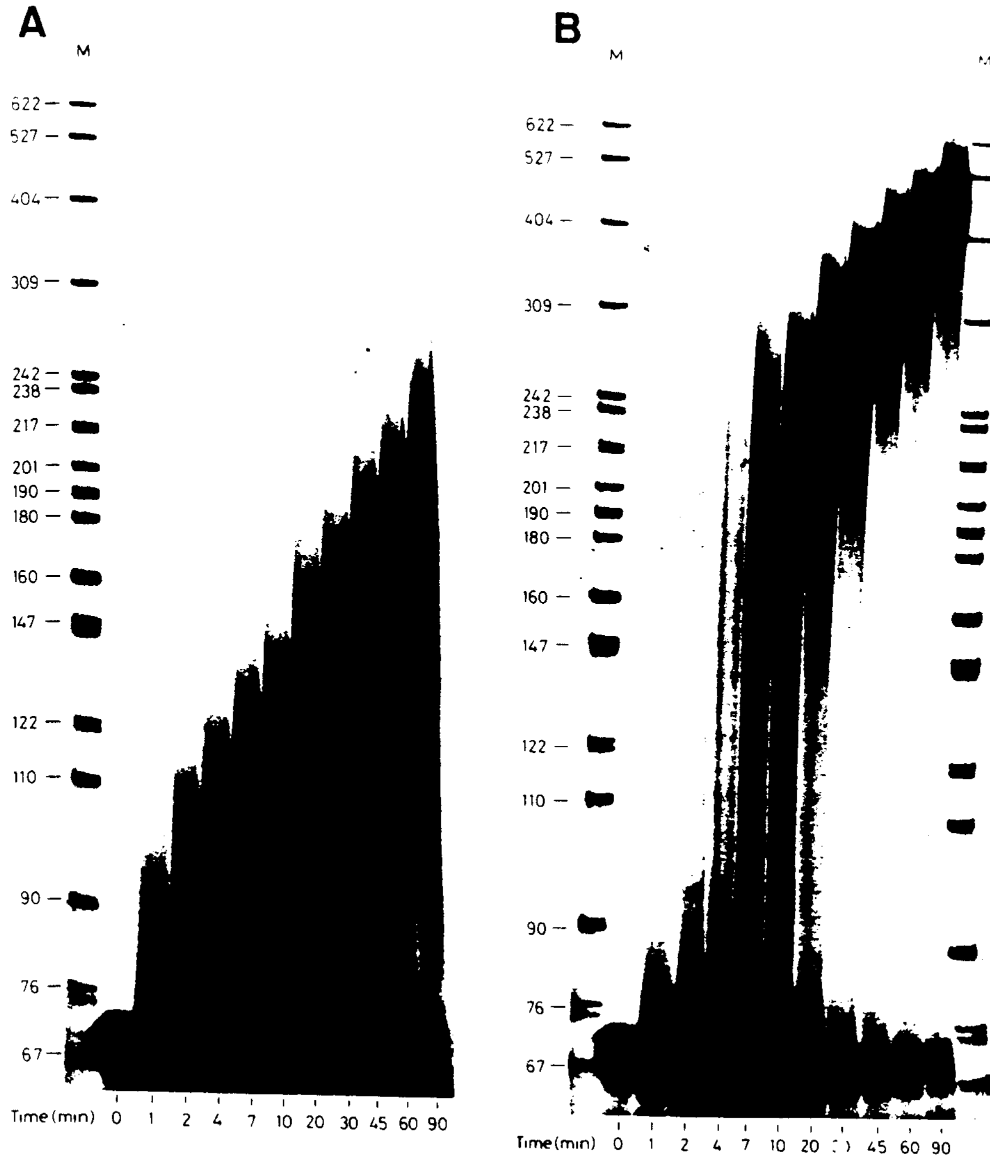


Figure 4. Kinetics of Polyadenylation in the Presence or Absence of PAB II

Two polyadenylation reactions 12-fold larger than the standard reaction were set up. All components except poly(A) polymerase were assembled on ice. The mixtures were prewarmed for 2 min to 37°C, and the reactions were started by the addition of poly(A) polymerase. Aliquots of 25 µl were withdrawn into SDS-containing proteinase K digestion buffer at the times indicated. RNA was purified (see Experimental Procedures) and analyzed on two long 5% polyacrylamide gels. (A) Kinetics of polyadenylation in the absence of PAB II. (B) Kinetics in the presence of PAB II; the reaction mix contained 340 ng of PAB II. Sizes of DNA markers (in nucleotides; lanes M) are given on the left.

PAGE D

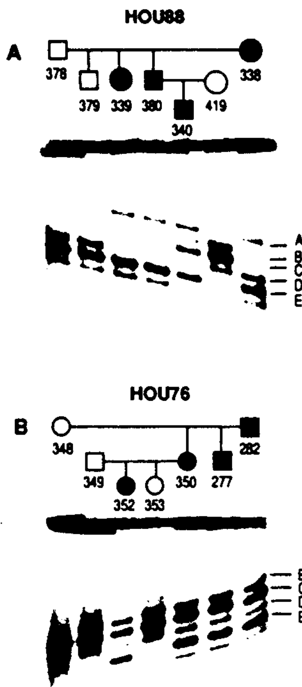


Figure 2. Detection of Three Alleles with the Marker RM11-GT in CMT Patients

(GT)$_n$ genotypes obtained by PCR analysis were scored as described in the legend to Figure 1. [illegible]. Shadow bands that differ from the primary bands in size by multiples of 2 bases are invariably seen with dinucleotide repeat polymorphisms; however, even without special precautions it is possible to read the genotypes unambiguously (Weber, 1990). (A) represents a nuclear family [illegible] (GT)$_n$ alleles. [illegible] (B) shows inheritance of three alleles in CMT patients from a nuclear family of Ashkenazic Jewish descent, in contrast to the other families, which are of French-Acadian descent.

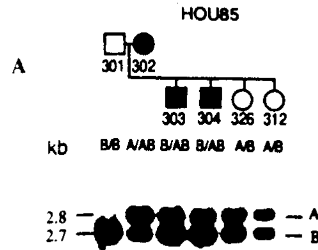


Figure 3. Southern Blot Analysis Demonstrates Dosage Differences of Polymorphic Alleles in CMT1A Patients

(A) Southern analysis of MspI-digested genomic DNA from a nuclear family (HOU85) with the probe VAW409R3 (D17S122). Southern analysis was conducted on 5 μg of genomic DNA as described (Patel et al., 1990a). Squares and circles represent males and females, respectively.

Charcot–Marie–Tooth disease type 1A (CMT1A) was localized by genetic mapping to a 3 cM interval on human chromosome 17p. DNA markers within this interval revealed a duplication that is completely linked and associated with CMT1A. The duplication was demonstrated in affected individuals by the presence of three alleles at a highly polymorphic locus, by dosage differences at RFLP alleles, and by two-color fluorescence in situ hybridization. Pulsed-field gel electrophoresis of genomic DNA from patients of different ethnic origins showed a novel SacII fragment of 500 kb associated with CMT1A. A severely affected CMT1A offspring from a mating between two affected individuals was demonstrated to have this duplication present on each chromosome 17. We have demonstrated that failure to recognize the molecular duplication can lead to misinterpretation of marker genotypes for affected individuals, identification of false recombinants, and incorrect localization of the disease locus.

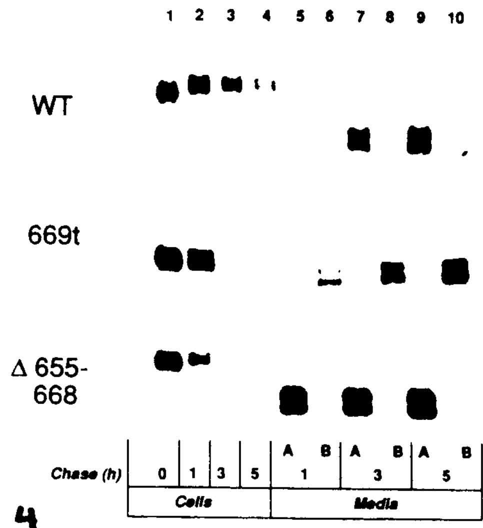
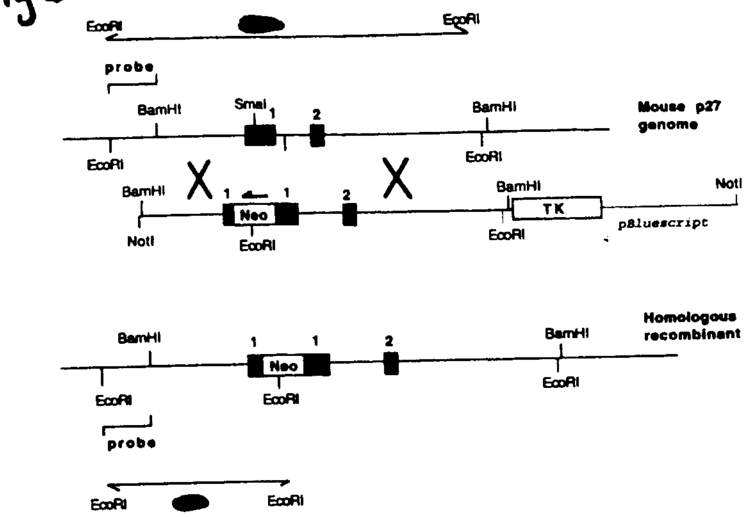


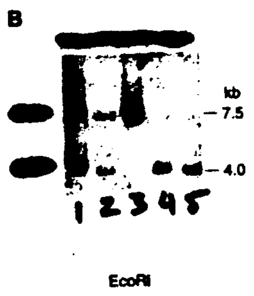
Figure 4 Pulse-Chase Analysis of SC Release

Wild-type pIgR (WT), pIgR669t, or pIgRΔ655–668 cells cultured on filters were metabolically labeled with [^{35}S]cysteine and chased in medium containing unlabeled cysteine for the times indicated. At each time point, cells and apical (A) and basolateral (B) media were harvested and immunoprecipitated with anti-SC antibody.

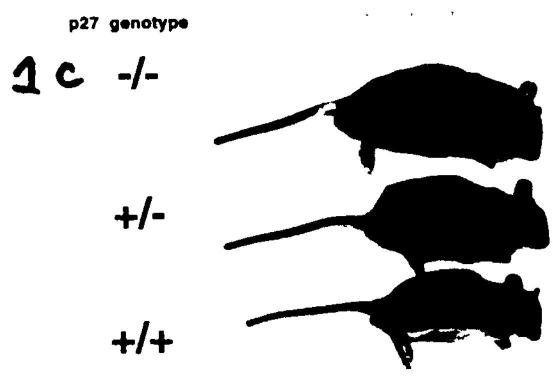
PAGE E

Fig 1





EcoRI



1D

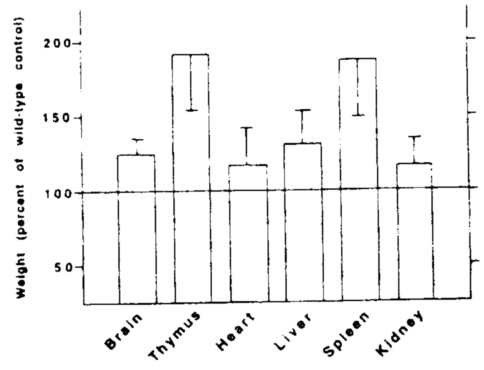


Figure 2. Exonuclease Activity of Purified p53 Protein

Gel electrophoretic measurements of exonuclease activity were carried out with the 30-mer deoxyoligonucleotide 5'-GACACTGGTCAC ACTTGGCTGCTTAGGAAT-3' (Foord et al., 1993). 3'-Labeling was performed with terminal transferase and [α-^{32}P]ddATP (lanes 1–4) and 5'-labeling with [γ-^{32}P]ATP and T4 polynucleotide kinase (lanes 5–10) as described under Experimental Procedures. The exonuclease activity was measured with ~1 ng p53, here purified from baculovirus-infected Sf9 cells by affinity chromatography with immobilized PAb421 and subsequent elution with 1 M KCl. The reaction products were analyzed on a 20% polyacrylamide gel containing 7 M urea. [illegible] lane 4 displays the same experiment as shown in lane 3, but in the presence of 5 mM GMP. Lanes 5–10 indicate product formation with the 5'-labeled substrate after 0, 2, 4, 8, 15, and 30 min incubation, respectively, at 37°C.

PAGE F

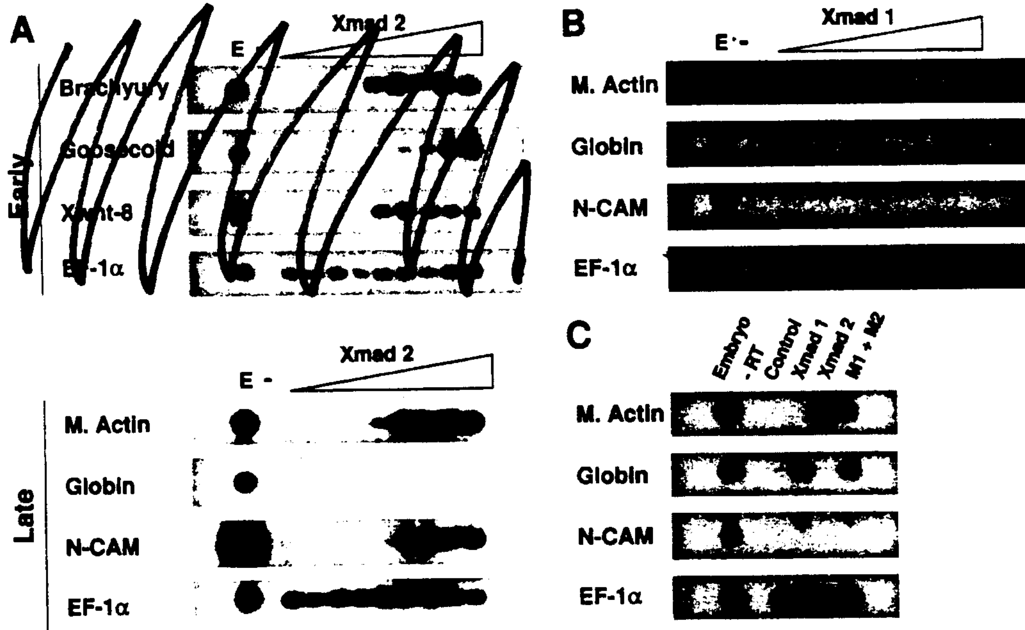


Figure 4. Dose-Dependent Induction of Mesoderm by Xmads

(A) Animal poles expressing different amounts of Xmad2 were cultured until either gastrula stage 11 (Early) or tadpole stage 38 (Late), and total RNA was harvested. RNA was analyzed by RT–PCR for the presence of the indicated transcripts. Xmad2 was expressed in a 2-fold dilution series from 2 ng to 15.6 pg. Xmad2 induces the expression of the different molecular markers beginning at about 125 pg of RNA in a concentration-dependent manner. [redacted] The markers and lanes are as described in the legend to Figure 3, except that the negative control is labeled with a minus sign.

(B) Xmad1 [redacted] Animal poles expressing different concentrations of Xmad1 were cultured until the tadpole stage 38, and total RNA was harvested. The concentrations of Xmad1 and the analysis is as described in (A). [redacted]

(C) Coexpression of Xmad1 and Xmad2 leads to formation of ventral and dorsal mesoderm. Animal caps expressing Xmad1 (2 ng), Xmad2 (2 ng), or Xmad1 and Xmad2 (M1 + M2; 2 ng of each) were cultured until tadpole stage 38, and total RNA was harvested. [redacted]. The analysis is as described in (A).

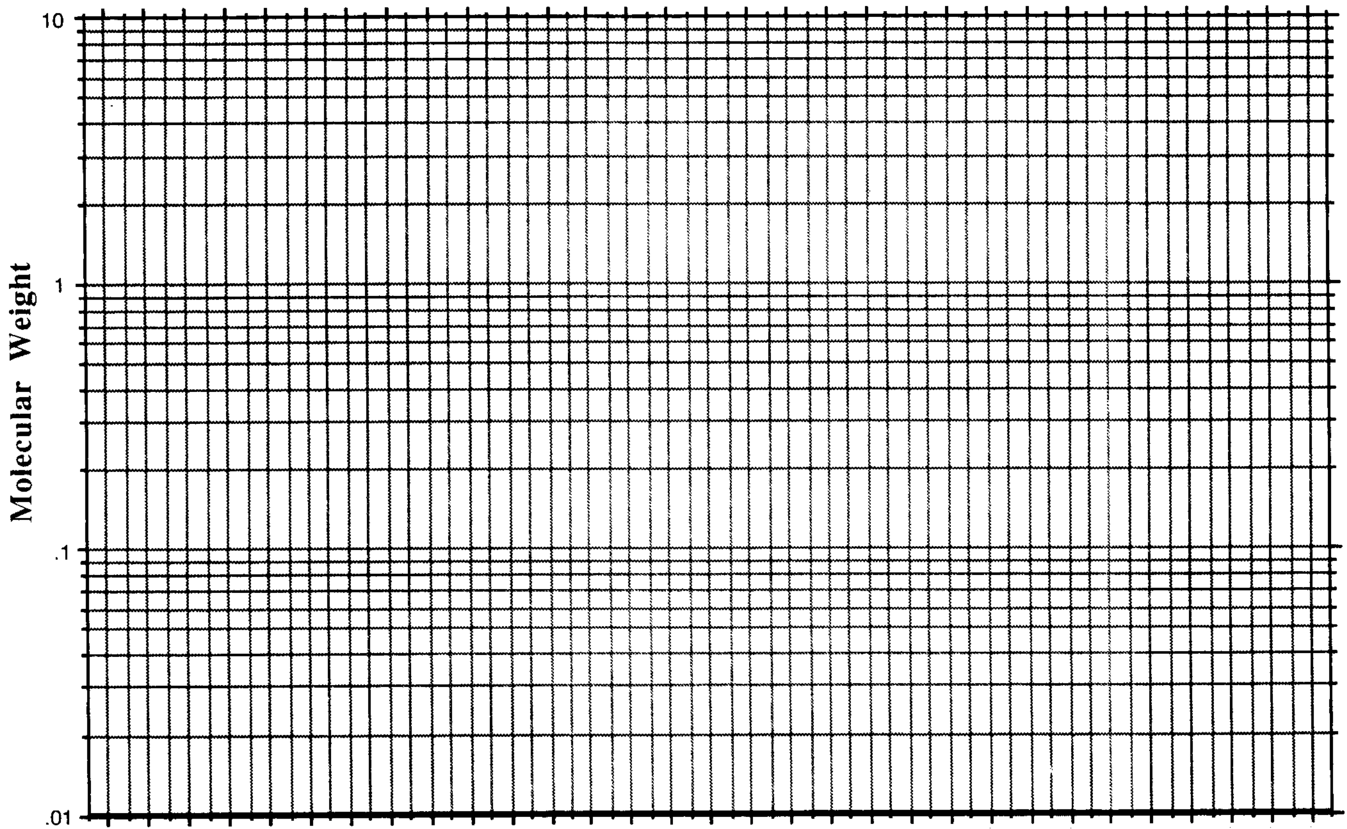
Questions based on figures from page A

8 pts.

1) Use the photo of the agarose gel and the graph paper provided here to calculate the molecular weight of the two bands in lane A as marked. Notice the molecular weights on the left side of 1.6, 1.0, 0.51, 0.40, 0.34, and 0.30 kb. You must draw a correct graph to get credit. Write your answers here:

Top band = _________

Bottom band = _________



8 pts.

2) Look at figure 2C and 2D. tk is a weak promoter and SV40 is the 3' UT portion that allows the CAT mRNA to be spliced and processed properly.

a) What is Oct-4? What is 6W?

b) What do we learn about the location of 6W?

6 pts.

3) Look at Figure 3 A - F. In E and F we see the plasmids used in this experiment. p6WtkCAT is the reporter plasmid from figure 2. pE1A is a piece of the Adenovirus that encodes the E1A protein. pCMV-Oct4 plasmid uses a strong viral promoter (CMV) to drive the expression of Oct4.

a) What do we learn about Oct-4 and E1A as demonstrated by these CAT assays (A-D)?

b) Why do you think panel A looks so funny?

Questions based on figures from page B

16 pts.

4) Figures 2 - 4 deal with the *Drosophila* gene *oskar* (*osk*). Look at all 3 figures before you answer any questions. These should be short answers.

a) When is *osk* transcribed in *Drosophila* (assume the positive control was positive for figure 2)?

b) What role does *osk* play in *Drosophila* development?

c) Briefly describe the localization of *osk* in wt embryos as shown in figure 4A - C. Stage 5 is at about 2.5 hrs while stages 2 and 3 are in the 0 - 2 hrs time period.

d) What would you predict the phenotype to be for figure 3 if this shows an in situ hybridization for *osk* of a stage 3 embryo?

8 pts.

5) What can you tell me about this epitope tag?

Questions based on figures from page C

6 pts.

6) Interpret these results from figure 4. PAB II is not a polymerase.

Questions based on figures from page D

8 pts.

7) Figures 2, 3 and the abstract are all related. What aspects of these two blots indicates the mutant genotypes? Notice that figures 2 and 3 are different kinds of blots so there might be more than one answer to this question.

10 pts.

8) Figure 4 shows the results of an experiment where we are monitoring a truncated protein called SC which is a mutant form of a plasma membrane receptor but it has had its transmembrane portion deleted. This mutant protein has about 800 amino acids while the full-length would have had about 1000 amino acids. WT means that of the remaining 800 amino acids, the sequence is wild type, while 669t has an amino acid substitution at position 669, and delta (D) 655-668 is a short deletion mutation. The assay takes advantage of the fact that the cells used in this study form a tight monolayer of cells which secrete some proteins up, away from the attachment side (apical), and other proteins are secreted down (basolateral) towards the attachment side. It is possible to collect these two separated media (apical and basolateral) because the cells are grown on a fine mesh filter instead of solid plastic and the cells and mesh can be lifted out of the culture disk at any time. Interpret these results.

Questions based on figures from page E

12 pts.

9) Figure 1 shows a series of related panels related to a strain of knockout mice with the p27 gene being tested.

a) What are the genotypes for mice 1 - 5 in panel B?

b) Describe the phenotypes from panel C and D and hypothesize an explanation.

c) Does this knockout mouse use the cell-specific mechanism for deleting a gene?

8 pts.

10) p53 has long been known to be involved in cancer as a tumor suppressor. In figure 2, we learn a new aspect about p53 - it is also an exonuclease. In this experiment, a 30mer oligonucleotide was end labeled either on the 3' end (left lanes) or the 5' end (right lanes) and incubated with 100% pure p53. Which end does p53 attack? Explain your answer. This one will take some time to think about but the answer should be short. Also, don't worry if you cannot read all of the legend, there is nothing important missing that I have not told you above, but if you want to try to read it you can.

Questions based on figures from page E

8 pts.

11) And the final question on this final is some what related to the paper we just finished on BMP and *tld*. Its great how our discussion of cloning the *tld* receptor was so close to what actually was done!

Ignore the top part (labeled EARLY) of panel A.

First, the frog (*Xenopus*) homolog for *tld* was cloned. Then in flies, the *tld* receptor was cloned and called *mad*. This figure was performed with the frog homologs to mad (or Xmad1 and Xmad2). Two Xmad genes were cloned. E marks the lane where an entire embryo was used. The wedges indicate how much of the appropriate gene was injected into the frog eggs.

There are various mRNAs being used here to figure out which part of the embryo is being induced with the two Xmads. M. actin = back muscles Globin = ventral tissue N-CAM = notochord/CNS

a) Interpret these results. You do **not** need to describe in detail what is in each panel, simply summarize your conclusions for all panels.

b) What do we learn about Xmads 1 and 2 that might lead to future research in flies and possibly humans?

Unrelated

c) Final easy two points to bring the total up to 100 pts. How many bands did you see on your zooblot from lab?

Now go out into the world and clone, sequence, etc. and publish your results so I will have test questions for future classes!