Exam #3

Molecular Biology Final Exam - spring 1997

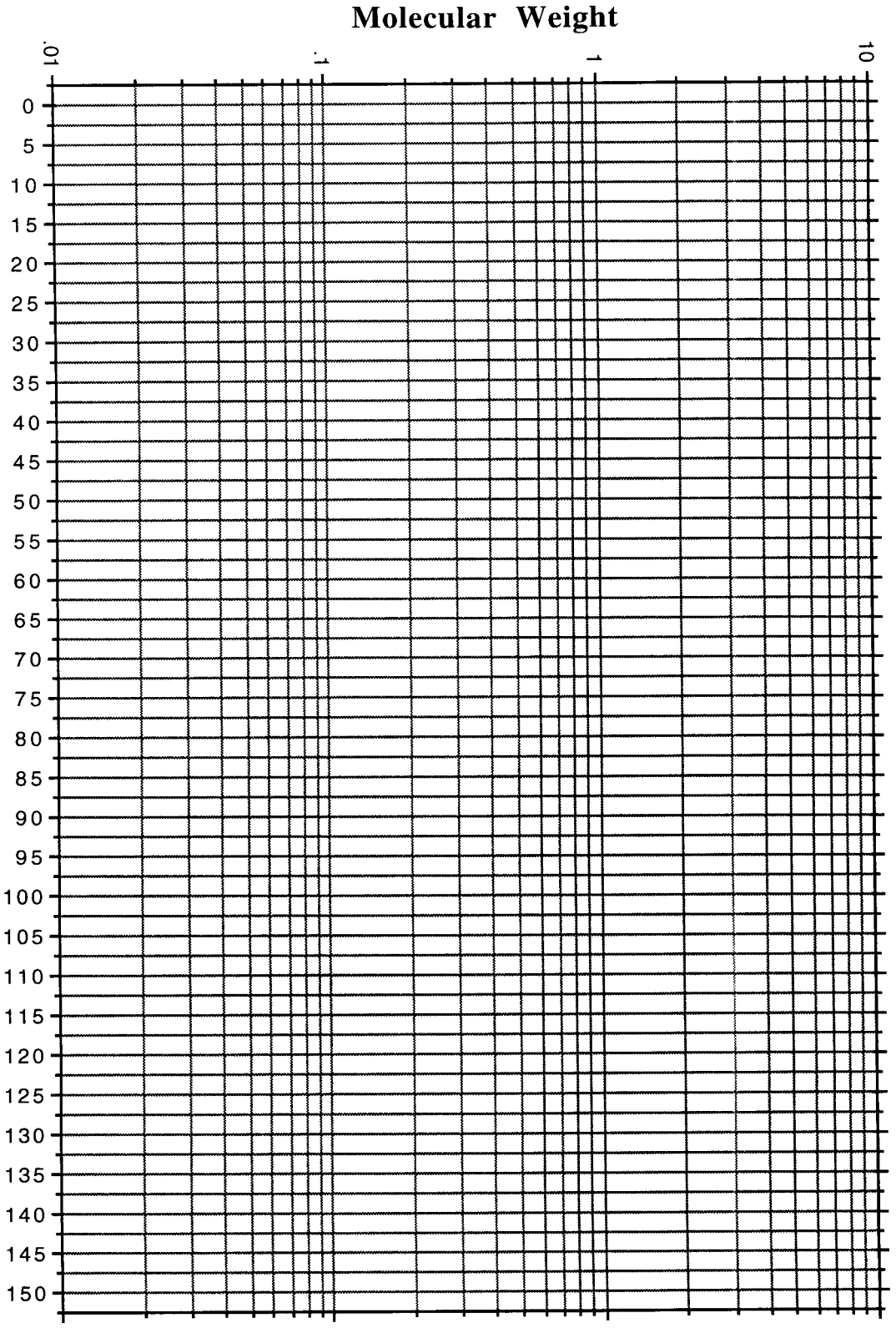
ALL EXAMS ARE DUE AT noon on MAY 10, 1997.

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 9. 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 **noon on MAY 10, 1997**. You may use a calculator, a ruler, and graph paper if you want. 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)?


Molecular Weight
.01
.1
1
10
0
5
10
15
20
25
30
35
40
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50
55
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65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150

8 pts.

1) Figure 1 has taken ER and Golgi membrane microsomes and analyzed the proteins as indicated. What can you determine about the proteins examined in this experiment?

8 pts.

2) Figure 2 shows the results of 3 experiments. KB cells were analyzed at various times (in hours) post-infection (hr p.i.) with either dl753 (a deletion mutant of a particular virus that is inactive inside cells) or rec700 (wt virus), or after treatment with Epidermal Growth Factor (EGF). The 170 kDa EGF-receptor (EGF-R) was analyzed after the membranes were treated with the detergent NP-40. DME is a particular kind of tissue culture medium, serum contains EGF, and cI21 is an anti-EGF-R antiserum.

What can you conclude from these data?

10 pts.

3) Figure 3 is merely to illustrate the kind of data one can get when using a particular P element to clone a novel gene. In this figure, we see that a reporter gene product is detected (light spots on the black background) in the photoreceptors of the fly (and nowhere else).

Use this strain of flies and the simplest method we have discussed in class to clone a gene that is expressed only in photoreceptors. You **must briefly outline** the steps you would need to perform in order to clone the wild-type allele of this gene. (There is more than one way to clone this gene but full credit will be given to the one method that is the most direct and requires the fewest steps. Partial credit will be given to all other methods.)

12 pts.

4) a) Calculate the molecular weight of the bands in lanes 1 and 3 of figure 4 A (the line indicates the location of the resolving gel). You must use the graph paper provided in this test in order to get full credit.

b) A soluble form of CD4 (sT4) was made, as well as two shorter forms of CD4 (V1V2 and V1). The soluble form of CD4 is comprised of four domains called V1 through V4. Above each ABC lane marker is a label that indicates which CD4 construct was used in the 4 experiments (a construct expressing V1 and V2 fused together was used twice). In all "A " lanes, an anti-CD4 antiserum was used for immunoprecipitation; in all "B" lanes, HIV gp120 was incubated with the indicated forms of CD4 and then an anti-gp120 antiserum was used for immunoprecipitation; in all "C" lanes, the anti-gp120 antiserum was used for immunoprecipitation in the absence of gp120. What can you conclude about HIV gp120 and CD4?

9 pts.

5) Figure 5 is an *in situ* hybridization of a fly egg before fertilization and it detects the mRNA from a single gene that is localized in the posterior end.

a) Hypothesize how a maternal mRNA can be localized to a particular region of an egg.

b) Design an experiment to determine how the mRNA can be localized to any discrete area of the egg. You do not need to give any volumes of reagents, just **OUTLINE** how you would design an experiment to determine the mechanism for an mRNA to stay in one region and not diffuse across the egg

8 pts.

6) Beta tubulin is half of a dimer (α/β dimer) that helps form microtubules. From these immunofluorescence data, what can you conclude about the role of microtubules in organelle structure?

8 pts.

7) a) From figure 7, what can you conclude about this promoter's response to the transcription factor RBP3 (RBP3 expression is under control of the CM viral promoter in this experiment.)

b) What is the obvious next experiment to do if you want to define the promoter even more?

8 pts.

8) What can you conclude from the data concerning RBP3 in figure 8?

10 pts.

9) a) What can you conclude about the location of the protein *noggin* ?

b) What can you conclude about the subcellular location of W1 during translation and later, when W1 finally reaches its proper subcellular location?

9 pts.

10) Tell me the name of the cDNA that has the accession number **M94130**. What organism is it from and in what journal was the sequence first published? In case you have not memorized the email method of doing this, you may use these directions:

1) Send a message to the following address:
retrieve@ncbi.nlm.nih.gov

2) Send a message that has the following format:

DATALIB[space]genbank
BEGIN
(Enter in the accession numbers for the sequence you found in the search above. Put only one accession number per line.) Hit return twice and then send the email.

10 pts.

11) Answer the following questions about the yeast 2-hybrid system:

a) What controls do you need to perform when cloning a cDNA by this method?

b) List all of the parts of each plasmid used for this method. You do not need to give me the particular amino acids, just the functional domains that we discussed in class.

c) Which of the two vectors/functional domains is used to make the genomic or cDNA library?

d) Where within the yeast cell must the resulting fusion proteins go?

e) How are these proteins targeted to the proper subcellular location?

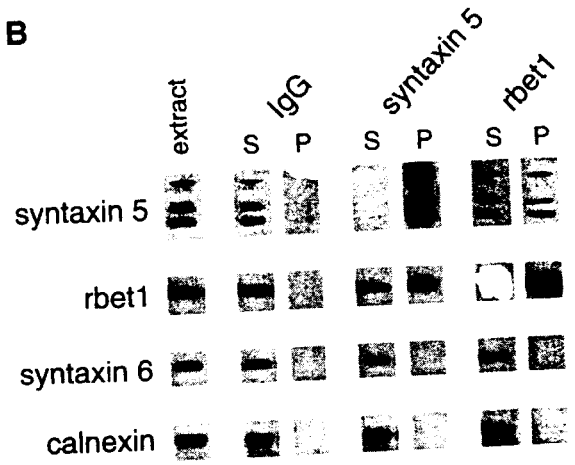


Figure 1. [redacted] Complex

(A) Rat liver membrane extract was fractionated by centrifugation through a 9.9% to 31.5% glycerol velocity gradient, and sequential fractions were analyzed for the presence of syntaxin 5 and rbet1 by electrophoresis and immunoblotting. Arrows indicate the mobilities of marker proteins.

(B) Immunoprecipitations were carried out as detailed in Experimental Procedures, and the resulting supernatants and pellets (S and P) analyzed by electrophoresis and immunoblotting. Labels along the top correspond to the antibodies used for immunoprecipitation, while labels along the left indicate the antibodies utilized for immunoblotting. The leftmost column labeled "extract" represents the starting membrane extract prior to addition of reagents or incubations. Pellets were resuspended in one-tenth the volume of the starting extract and supernatant. Thus, quantitative precipitation would result in a pellet band 10× more intense than in the starting extract, and 10% efficient precipitation would result in a pellet band of roughly equal intensity to the starting extract and partially depleted supernatant.

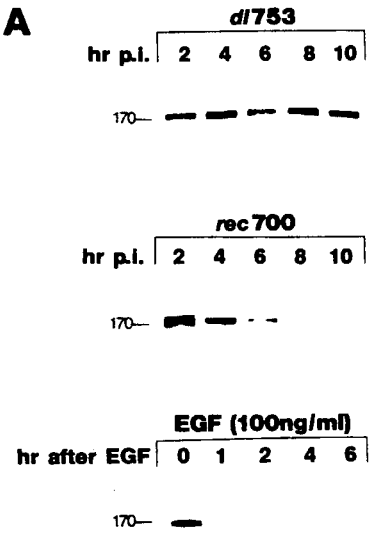


Figure 2. Analysis of EGF-R [redacted] in KB Cells following Infection with *dl753* or *rec700*, or Stimulation with EGF

(A) KB cells were pulse-labeled with L-[^{35}S]cysteine for 1 hr followed by incubation with DME supplemented with 5% horse serum for 3 h. Cells were then either infected with *dl753* or *rec700* in serum-free medium, or stimulated with EGF following an additional 1 hr pre-incubation in serum-free medium. Cells (10^7) were collected at the intervals indicated, and NP-40 extracts were immunoprecipitated using cl 21 antiserum. A ten-fold excess of unlabeled L-cysteine was included in all incubations after the initial pulse label.

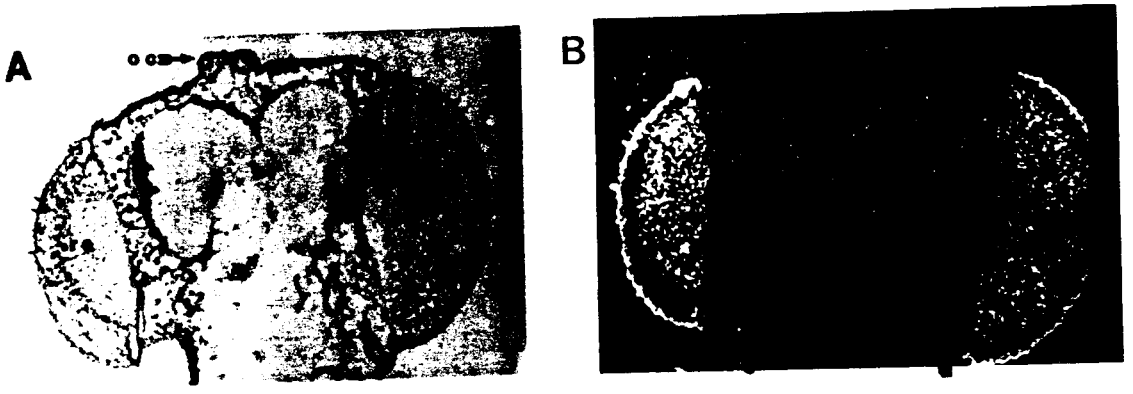


Figure 3. Spatial Localization of [redacted] RNAs by In Situ Hybridization to Tissue Sections of Adult Fly Heads

Frozen sections of adult fly heads were hybridized to (A, B) [redacted] DNA probes nick translated with ^{3}H-labeled dNTPs. A, C, and E, bright-fields; B, D, and F, dark-fields. re, retina; la, lamina ganglionaris; me, medulla; oc, ocelli; br, brain. In the dark-field images (B, D, and F), some light scattering by the cuticle is seen. [redacted] appears to be transcribed in [redacted] photoreceptor cells. [redacted]

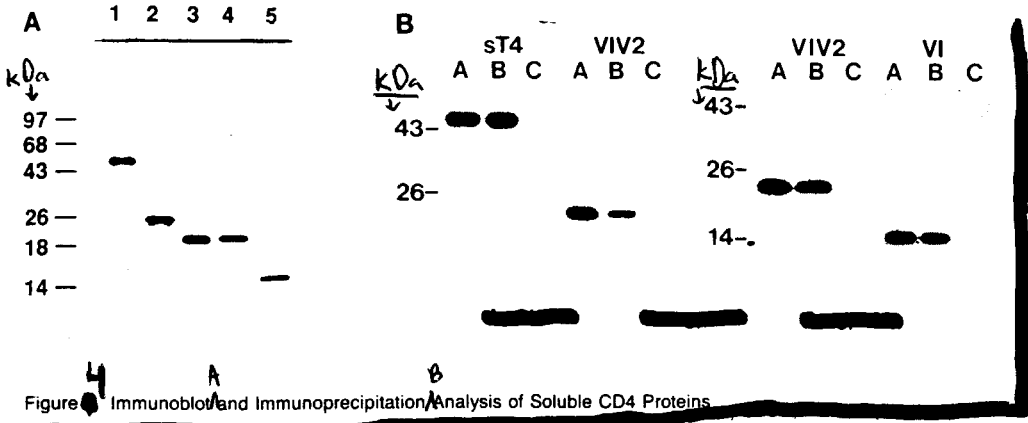


Figure 5 Immunoblot and Immunoprecipitation Analysis of Soluble CD4 Proteins

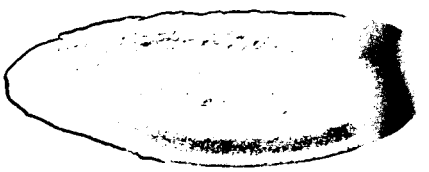


Fig 5

which can be experimentally reproduced by exposing cells to the microtubule depolymerizing drug, nocodazol

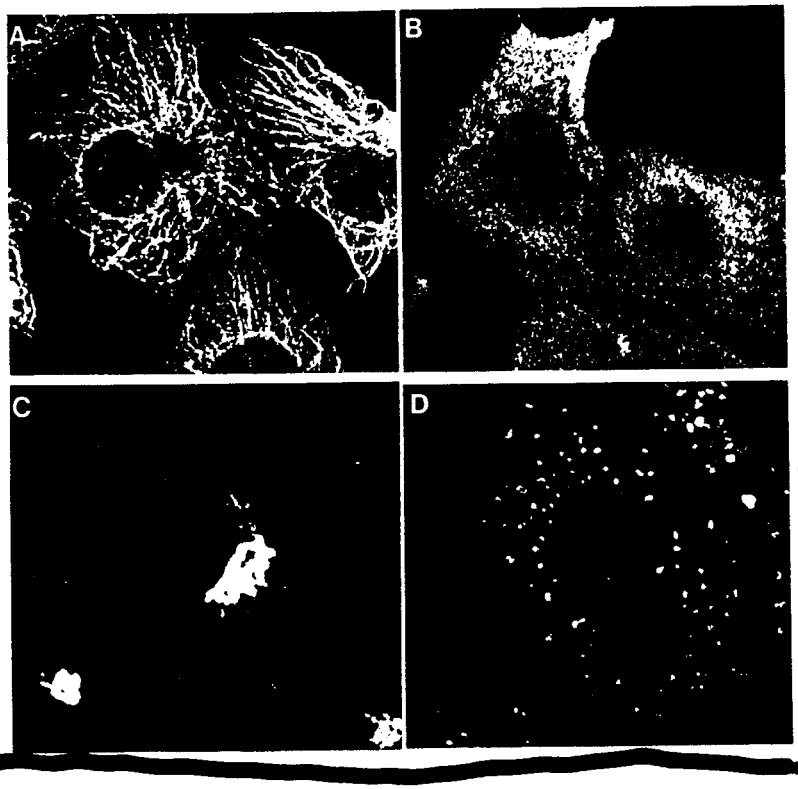
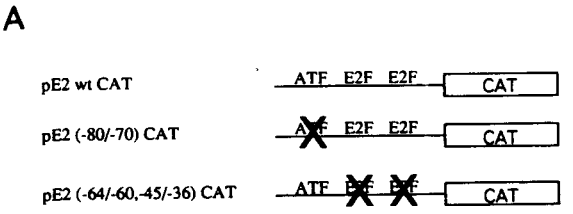


Figure 6. Treatment of Cells with Nocodazol

BHK cells were treated with 10 μM nocodazol for 4 hr prior to fixation and processing for immunofluorescence. Staining for β-tubulin confirmed that following treatment, the normal microtubular organization (A) had been completely disrupted (B). The distribution of anti-TCP-1 staining was localized both before and after (D nocodazol.



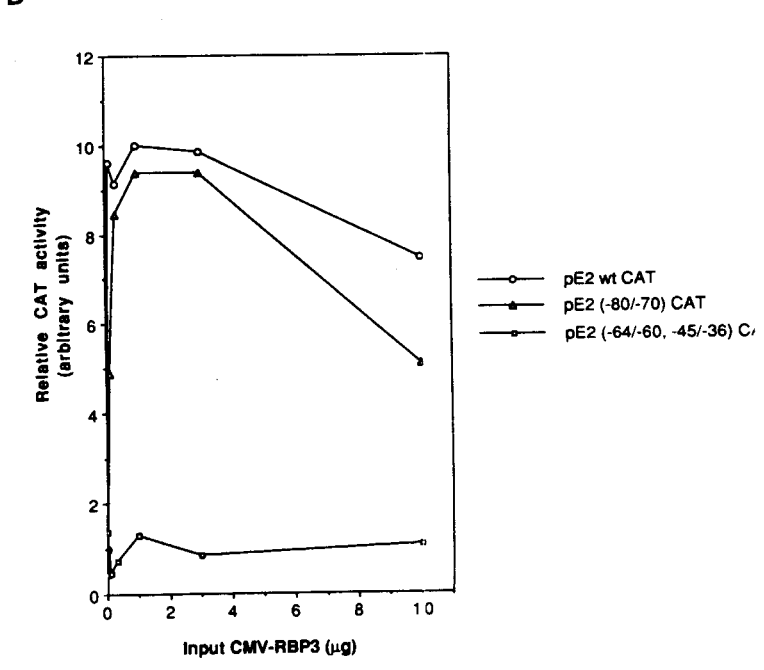


Figure 7. Transcriptional Activation by RBP3

(A) Schematic organization of the reporter constructs pE2 (wt) CAT, pE2 (-80/-70) CAT, and pE2 (-64/-60,-45/-36) CAT.

(B) The response of the three reporters to input pCMV-RBP3 plasmid has been assessed in T98G cells. These were transiently transfected in duplicate with 10 μg of either pE2 (wt) CAT, pE2 (-80/-70) CAT, or pE2 (-64/-60, -45/-36) CAT; 0, 0.1, 0.3, 1, 3, or 10 μg of pCMV-RBP3 (as indicated), . Relative CAT activity refers to unit CAT activity

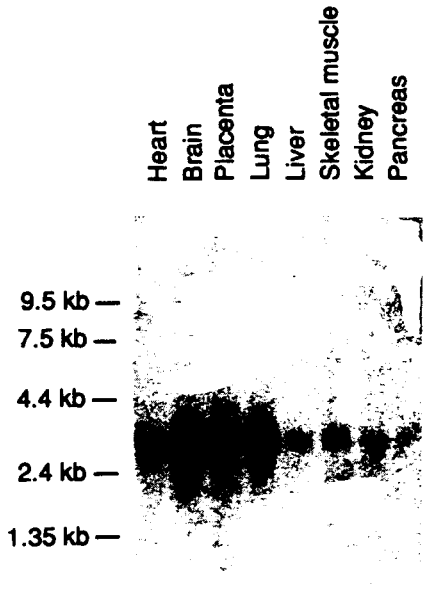
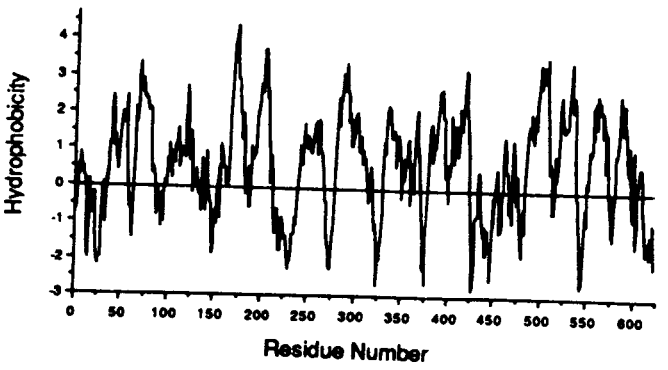


Figure 8 Northern Blot Analysis of *RBP3* mRNA Isolated from Human Tissues

A human tissue Northern blot (Clontech) was probed with a 2.0 kb *RBP3* cDNA probe (nucleotides 498–2517). Each lane contains poly(A)$^+$ RNA from the indicated tissues. Size markers are indicated on the left in kilobases.

9B



Nucleotide and Deduced Amino Acid Sequence of the W1 cDNA and Hydropathy Plot

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                                    Eco RI
  1:MetAspHisSerGlnCysLeuValThrIleTyrAlaLeuMetValPheLeuGlyLeuArgIleAspGlnGlyGlyCysGlnHisTyrLeuHisIleArgProAlaProSerGluAsnLeu
  1:ATGGATCATTCCCAGTGCCTTGTGACTATATATGCTCTGATGGTCTTCTTGGGACTTAGAATAGACCAAGGGGGTTGCCAACATTATCTGCACATCAGACCGGCTCCTAGTGAAAACCTA

 41:ProLeuValAspLeuIleGluHisProAspProIleTyrAspProLysGluLysAspLeuAsnGluThrLeuLeuArgThrLeuMetValGlyHisPheAspProAsnPheMetAlaThr
121:CCACTGGTGGACCTTATTGAGCACCCGGATCCCATCTATGATCCCAAGGAGAAGGATCTTAACGAGACCTTGCTGAGGACTTTAATGGTTGGACACTTTGACCCCAACTTTATGGCCACC
                                    Bam HI
 81:IleLeuProGluGluArgLeuGlyValGluAspLeuGlyGluLeuAspLeuLeuLeuArgGlnLysProSerGlyAlaMetProAlaGluIleLysGlyLeuGluPheTyrGluGlyLeu
241:ATCCTGCCAGAGGAGAGACTTGGAGTGGAGGACCTTGGGGAGTTGGATCTCCTTCTTAGGCAGAAGCCCTCGGGGGCAATGCCAGCGGAAATCAAGGGACTGGAGTTTTACGAGGGGCTT

121:GlnSerLysLysHisArgLeuSerLysLysLeuArgArgLysLeuGlnMetTrpLeuTrpSerGlnThrPheCysProValLeuTyrThrTrpAsnAspLeuGlyThrArgPheTrpPro
361:CAGAGCAAAAAGCACAGACTGAGCAAGAAACTCAGGAGAAAGTTGCAGATGTGGCTCTGGTCCCAGACCTTCTGTCCTGTCCTTTACACATGGAATGACCTAGGGACCAGGTTTTGGCCT
          <- 5.5
161:ArgTyrValLysValGlySerCysTyrSerLysArgSerCysSerValProGluGlyMetValCysLysAlaAlaLysSerMetHisLeuThrIleLeuArgTrpArgCysGlnArgArg
481:CGCTATGTGAAAGTAGGGAGCTGCTACAGTAAGAGGTCTTGTTCTGTGCCAGAGGGCATGGTTTGCAAAGCTGCCAAGTCTATGCATTTGACCATCTTAAGGTGGAGATGTCAACGCAGG

201:ValGlnGlnLysCysAlaTrpIleThrIleGlnTyrProValIleSerGluCysLysCysSerCys stop
601:GTTCAGCAGAAGTGTGCGTGGATAACCATTCAGTACCCTGTCATTTCCGAGTGCAAATGCTCATGCTGAGACTCTTGGACTAATGCAAAAAGACAGTAGCTTCATGGTTCAAGCTTCATG
                                                                    Hind III
721:TTATATGCACTGTAATATGTAGAAATGTATATGTGTGTATATATGGCATTGGTCTAAATTACTATTAAAAGGTCAGTATTATTCTTTTAAATAACCAGTGTCTACTGTATTTCCAACACT

841:ATTATCCTGGTTGTGTTTTATTTTAATAATATTATTATTATTTTTTTTTTGCCTAATGTATCTCTATTTATATCCAAAAAAAGAGCACTTCGCTTGGCGAAGCATTTTTTTTAAAGAAA

961:AAAAAAACAAATTTAATAGTTTAATAATATAGAAGCATTTTTTTCCTTTAATGGAAAATGTGCCTTTTTTTGATGGACCTCAAAAAAAAAATGAATAAAAACCAGAGCAAGATATAATTT

1081:TCAGTTTATTGTACATAGAAAGAGAACACATTTTGAAAGATGAAAAATTTTACTCTTGTAAATGAGAACTATCTGCTATTTATTCTTTTATTTTTTTTTCCTCCCCCTGTAGAGTGCAG

1201:AATAAAAGTAAACCACTAAAATATTAAAAAAAAAAAAAAA
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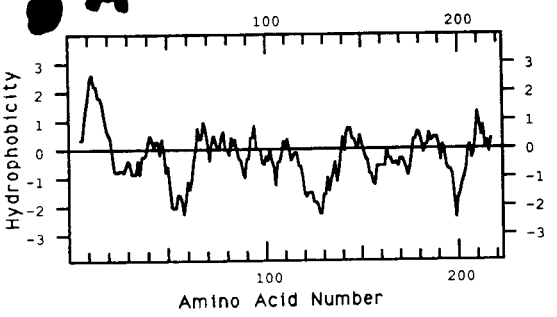

9A

Figure 9 Nucleotide Sequence of *noggin* cDNA and Hydropathy Plot of Predicted noggin Polypeptide

(A) Nucleotide sequence of *noggin* cDNA. The complete nucleotide sequence and the predicted amino acid sequence encoded by *noggin* cDNA are shown. A potential N-linked glycosylation site (asparagine at amino acid number 61) is indicated by an asterisk. Potential polyadenylation sequences (AATAAA) are underlined. The 3′ end of exonuclease clone 5.5, which was used as a template for RNAase protection assay, is underlined.

Hydropathy plot of predicted noggin polypeptide. The hydropathy of predicted noggin polypeptide was plotted by the method of Kyte and Doolittle (1982).