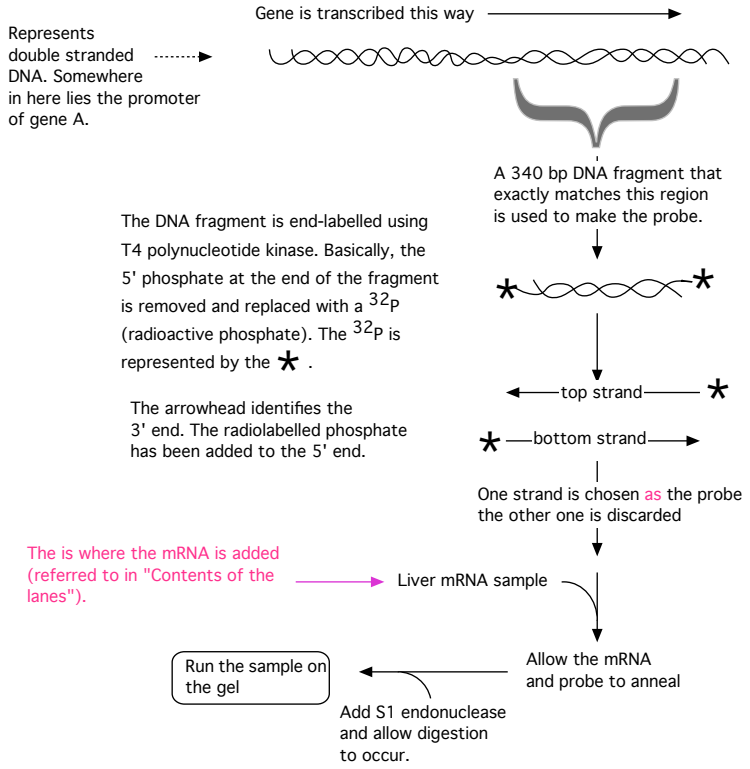
Name _____ KEY _____ 11:00 am class Spring 2009

In this experiment you are studying the promoter region and the regulation of a gene called ***A***. You do not know very much about this gene. Gene ***A*** is a gene that is expressed only in the mammalian liver.

You are studying a medication (compound 5567679) that boosts liver function in alcoholics. It seems to act through gene ***A***.

The mRNA used in lanes 4-8 comes from mice that consumed an increasing dose of compound 5567679.

You are going to use S1 protection to characterize some of the effects of 5567679. The procedure below has been followed.



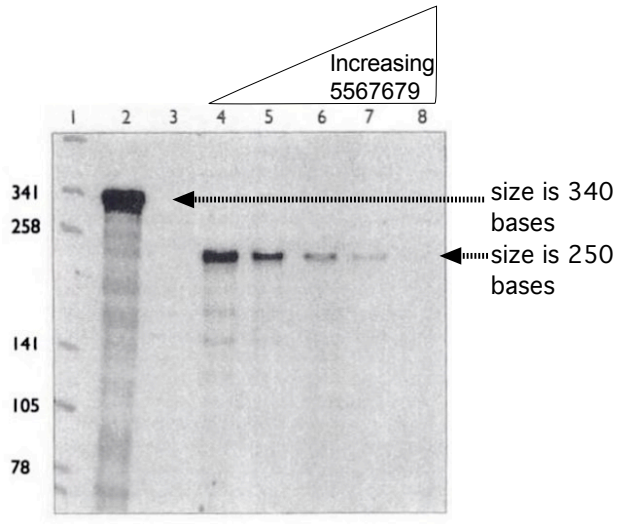


Photo of gel was borrowed from *A laboratory guide to RNA isolation, analysis and synthesis*. Edited by Paul A. Krieg.

The probe is derived from mouse genomic DNA. It is known that the probe fragment covers the transcription start site (tsp, +1) but the exact position of the transcription start site has not yet been determined.

The S1 digestion products are loaded onto a gel and electrophoresed. The figure on the right shows an autoradiograph of the gel.

Contents of the lanes

Lane 1 size standards

Lane 2 Mock digested. *Mock* means "fake". In this context it means that Lane 2 is identical to lane 4 except that endonuclease S1 was not added.

Lane 3 mRNA from yeast is added but no liver mRNA is added.

Lane 4-8 mRNA from liver is added.

In lane 4 - from animals that did not receive compound 5567679

In lane 5 - from animals that received 1 unit of compound 5567679 per day

In lane 6 - from animals that received 2 units of compound 5567679 per day

In lane 7 - from animals that received 3 units of compound 5567679 per day

In lane 8 - from animals that received 5 units of compound 5567679 per day

Essay Question 1:

How long is the probe?

Answer: 340 bp

Where is the transcription start site?

*Answer: 250 bp from the 5' end of the top strand probe. OR 250 bp from the * on the top strand probe.*

How long is the protected product?

Answer: 250 bases

Describe one effect of compound 5567679 that is apparent in the gel.

Answer: It reduces the expression of Gene A.

What is the purpose of lane 2? Give a comprehensive explanation. Don't give me just a 1-3 word answer.

Answer: This is a control (the students might but do not have to say negative control). This lane serves multiple purposes.

1) It confirms that the probe has been made correctly since it is supposed to be 340 bp.

2) It confirms that the probe has not broken down (degraded) because of some unfortunate accident. This can happen because of a mistake in handling.

3) It displays the undigested probe on the gel so that you can compare your bands to it.

What is the purpose of lane 3? Give a comprehensive explanation. Don't give me just a 1-3 word answer.

Answer: This is also a control (it might also be called a negative control but this is not required).

This control confirms that your enzyme is working and that it will destroy any probe that is not protected by base-pairing to a liver mRNA (or some other nucleic acid).

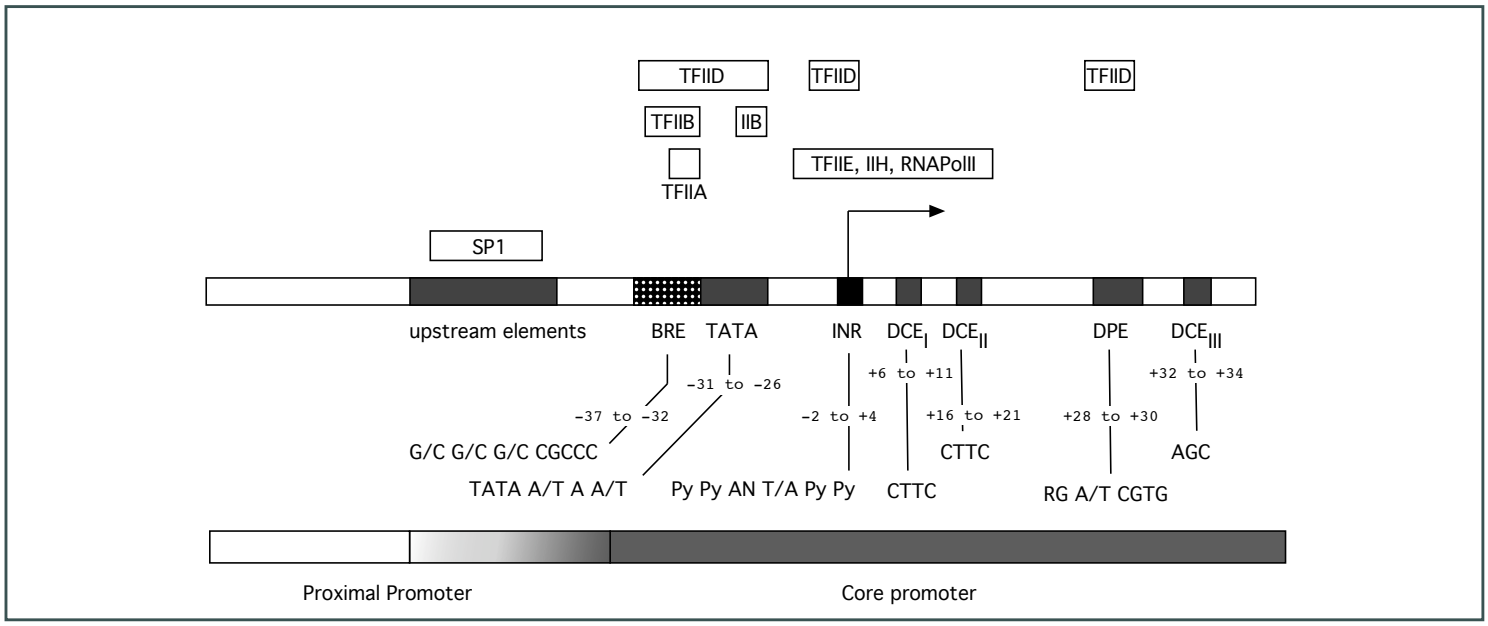
It also confirms that you do not contaminating nucleic acid that is protecting your probe (not confirmed for you liver mRNA sample. It is very hard to confirm this).

If you were to see a band in this lane then it would indicate that your S1 endonuclease was not functional or that some contaminating RNA or DNA was protecting your probe.

This control is able to do this because the probe is specific to a gene from a mammal and therefore, it should not have anything to base pair to in the sample of yeast mRNA. As a result, the S1 endonuclease should destroy all of the probe.

Essay Question 2: *Please describe an RNA polymerase II core promoter. Tell me everything that you know about such promoters. Identify specific sequences and the proteins that interact with (bind, recognize) these sequences.*

Answer:



This figure could be used. All of these elements should be mentioned. They do not have to mention the proximal promoter or core promoter parts at the bottom of the figure.

The students can refer to the sequence elements by name. They do not have to provide the nucleic acid sequence (but do not subtract points if they spell out the sequence and make a mistake).

They should also mention the SP1 binding site by name even though it not specifically labelled in this particular figure.The students can also mention the MTE (motif ten element). If they do give them points for it but if they don't do not subtract points.

At the top are the places that proteins interact. This is a bit different than binding. They don't have to explicitly name those particular interactions.

The interactions that they should name are:

The students should specifically say the TATA box is recognized/bound by TBP (a part of TFIID).

In addition the students should say that the INR and DPE are recognized by TAFII250 and TAFII150 (also parts of TFIID).

They should say that TFIIB interacts with BRE.

It is also required that they say that the minimum functional requirement of an RNA polymerase II promoter is that it identify the position where transcription should begin and the direction that it should proceed (It does not actually have to specify that transcription occur although it might).

In all cases the students can use the expanded version of the abbreviation or the abbreviation.

Essay Question 3: *Tell me everything that TFIID does. Be very thorough.*

Answer:

TAFII250 and TAFII150 are part of TFIID. These two recognize/bind INR and DPE.

TAFII250 is a histone acetyl transferase that can acetylation the lysine residues of histones.

TAFII250 is also a protein kinase that phosphorylates itself and TFIIF, TFIIA and TFIIE. This is thought to modulate activity of the initiation complex.

TBP is part of TFIID. It recognizes/binds the TATA box.

When TBP binds the TATA box it causes the DNA to bend sharply. (They might also say that the bending causes some melting and that it can push a nucleosome out of the way - give them credit for this but do not subtract points if they do not mention these).

TFIID is the first component of the pre-initiation complex to bind the promoter (except for promoters that only have an SP1 binding site and then it is the second). The presence of TFIID helps the other TFs of the pre-initiation complex to bind (students might say that this more directly by naming individual TFs).

Essay Question 4: *A mutation in one gene knocks out transcription of most mRNA genes, all tRNA genes, and all rRNA genes. Based on the lecture or your reading material, what does this gene encode (give me the most likely thing)? Why does some mRNA expression remain? This is a very-very-short-answer question.*

They can use Answer 1 for the first part and Answer 2 for the second part or vice-versa.

Answer 1: TBP.

Some mRNA transcription could remain because it is dependent on TRF1 (TBP-related factor) or TLF (TBP-like factor) or TFTC (TBP-free TAFII complex). They only need to name one of these.

For extra credit, please provide a second reasonable answer for this question. The extra-credit question is also a very-short-answer question. Clearly mark the extra credit part as extra credit.

Answer 2: The answer could be any only of the shared RNA polymerase subunits. Some (eg. RPB5) are shared amongst all eukaryotic RNA polymerases (see the table below for other specific examples). The students can either specifically name the subunit or they can explicitly refer to this concept.

Table 10.2 Human and Yeast RNA Polymerase II Subunits

Subunit	Yeast Gene	Yeast Protein (kD)	Features
hRPB1	*RPB1*	192	Contains CTD; binds DNA; involved in start site selection; β′ ortholog
hRPB2	*RPB2*	139	Contains active site; involved in start site selection, elongation rate; β ortholog
hRPB3	*RPB3*	35	May function with Rpb11 as ortholog of the α dimer of prokaryotic RNA polymerase
hRPB4	*RPB4*	25	Subcomplex with Rpb7; involved in stress response
hRPB5	*RPB5*	25	Shared with Pol I, II, III; target for transcriptional activators
hRPB6	*RPB6*	18	Shared with Pol I, II, III; functions assembly and stability
hRPB7	*RPB7*	19	Forms subcomplex with Rpb4 that preferentially binds during stationary phase
hRPB8	*RPB8*	17	Shared with Pol I, II, III; has oligonucleotide/oligosaccharide-binding domain
hRPB9	*RPB9*	14	Contains zinc ribbon motif that may be involved in elongation: functions in start site selection
hRPB10	*RPB10*	8	Shared with Pol I, II, III
hRPB11	*RPB11*	14	May function with Rpb3 as ortholog of the α dimer of prokaryotic RNA polymerase
hRPB12	*RPB12*	8	Shared with Pol I, II, III

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Name _____KEY_____

Multiple Choice section of the exam.

1. ***The promoter used in the rRNA genes***
   a. is internal to the transcribed part of the gene.
   b. consists of 5 related sequences.
   c. requires TBP for activation. *

2. ***Which statement best describes a promoter for a tRNA gene?***
   a. A TATA box that is sometimes accompanied by an INR, a GC box, and a DPE
   b. Two boxes that are both internal to the transcribed region of the gene *
   c. Two boxes, one of which is upstream of +1 and one of which overlaps +1.

3. ***Which statement best describes the interaction between TFIIIB (three B) and tRNA promoters?***
   a. TFIIIB contains TBP and helps position RNA polymerase III over +1. *
   b. TFIIIB is the unit that by itself binds the tRNA promoter and recruits RNA polymerase III.
   c. TFIIIB binds both RNA polymerase III and RNA polymerase I promoters

4. ***In epitope tagging***
   a. a DNA sequence that encodes the amino acid sequence recognized by an antibody is inserted into a gene. *
   b. a DNA sequence that encodes an antibody is inserted into a gene.
   c. part of the gene under study is inserted into a gene that encodes an antibody

5. ***Which is a description of a Quantitative S1 Nuclease Protection assay?***
   a. A primer is allowed to anneal to an mRNA. Reverse transcriptase is used to synthesize cDNA using the mRNA as a template. S1 endonuclease is added and degrades all single-stranded nucleic acid. The double-stranded DNA:RNA hybrid survives.
   b. The DNA is digested with endonuclease S1, and then *in vitro* transcription is performed. The polymerase falls off the end of the DNA at the S1 site. The S1 cut acts as a transcription termination site.
   c. A DNA fragment is end labeled. One DNA strand is annealed to mRNA from a gene and the resulting heteroduplex is digested with S1 endonuclease. *

6. ***A Quantitative S1 Nuclease Protection Assay can determine what?***
   a. the relative abundance of a single mRNA, but only if no other mRNAs are present
   b. the relative abundance of a single mRNA in a complex mixture that contains a great many different mRNA species *
   c. whether or not a protein binds a specific DNA sequence

7. ***Which of the following is an internal control? We are studying transcription from promoter A. In our experiments, we make a series of mutations in the DNA containing promoter A. Some of these mutations eliminate transcription from promoter A. An internal control would be:***
   a. In a separate tube, we also monitor transcription from the wild-type version of promoter A.
   b. In the same tube as the mutant promoter A DNA we include promoter B DNA. We also monitor the expression of promoter B even though we do not introduce mutations into it. *
   c. We include a tube in which the nuclear extract is added but the promoter A DNA is omitted.

8. ***A gel shift assay can***
   a. be used to determine the position of +1.
   b. be used to determine the abundance of a single mRNA even if a great many other RNAs are present.
   c. be used to determine if a protein binds to a DNA sequence. *

9. ***During the activation of a promoter recognized by RNA polymerase II, which is an acceptable order of binding to the core promoter?***
   a. TFIIA, TFIIB, TFIID, and then RNA polymerase and TFIIF
   b. TFIID, TFIIA, TFIIB, and then TFIIF and RNA polymerase *
   c. TFIIB, TFIID, RNA polymerase, TFIIF, and then TFIIA
   d. TFIID, RNA polymerase and TFIIF, TFIIA, and then TFIIB
   e. RNA polymerase and TFIIF, TFIID, TFIIA, and then TFIIB

10. ***For a DNA sequence to be considered to be a core promoter for RNA polymerase II it must at least***
   a. promote transcription when all parts of the pre-initiation complex are present.
   b. specify the direction, start site and amount of transcription that should occur.
   c. specify the direction of transcription and location where transcription should begin. *

11. ***TBP***
   a. binds the major groove of the TATA box; this bends the DNA sharply, this bending is a form of annealing
   b. binds the minor groove of the TATA box; this bends the DNA sharply, this bending is a form of melting *
   c. binds the INR promoter element and positions RNA polymerase over +1. It is TFIIB that melts the DNA.

12. ***TBP***
   a. phosphorylates the CTD domain of RNA polymerase II.
   b. is involved in the transcription of all mRNAs synthesized by RNA polymerase II.
   c. is involved in the transcription of tRNAs and rRNAs. *

13. ***Which statement best describes the recognition of the TATA box promoter element?***
a. TFIIB is thought to recognize this promoter element.
b. Within TFIID, only TBP is thought to be able to recognize this promoter element. *
c. Within TFIID, both TBP and TAFII250 are thought to be able to recognize this promoter element.

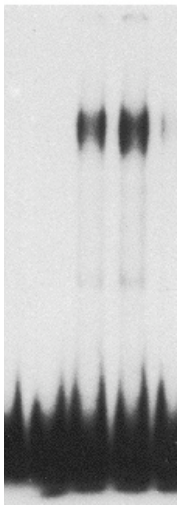
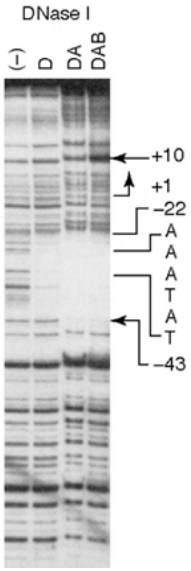
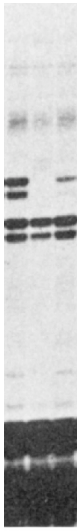
14. ***TRF1 (TBP-related factor 1)***
   a. can take the place of TBP in some RNA polymerase II pre-initiation complexes. *
   b. dimerizes with TBP to form TFd (double TBP), which recognizes promoters in yeast.
   c. used only in RNA polymerase I pre-initiation complexes.
   d. does not exist.

15. ***TFIIB***
a. binds the DPE element in place of TAF250/TAF150.
b. can at one of its ends bind TFIID and at the other end can bind TFIIF/RNA polymerase II. *
c. can bind BRE and then recruit RNA polymerase without the presence of TFIID.

16. ***Most transcription factors recognize a DNA sequence by interacting with***
   a. acetylated histones.
   b. the DNA after it has been denatured.
   c. parts of the nitrogenous bases through the major groove of the DNA. *
   d. parts of the nitrogenous bases through the minor grove of the DNA.

17. ***The bacterial protein Sigma***
   a. binds tightly to RNA polymerase and directly recognizes bacterial promoters. *
   b. is a negative regulator of transcription.
   c. is a part of TFIID in bacteria.

18. ***Which autoradiograph represents footprinting?***

A	B *	C	D
			

19. ***Gal4 is a***

a. mammalian transcription factor that belongs to the Type I hormone receptor group.
b. mammalian transcription factor that belongs to the Type II hormone receptor group.
c. yeast transcription factor that is a C4 Zinc-containing transcription factor. It binds a hormone response element.
d. yeast transcription factor that is a C6 Zinc-containing transcription factor. It binds as a dimer. *

20. ***Thyroid hormone receptor is a transcription factor that***

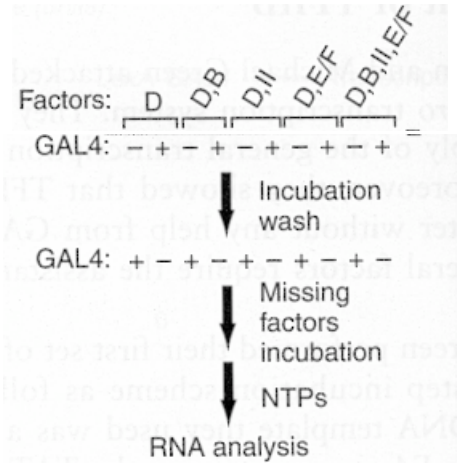
a. is found in the cytoplasm when it is not bound to its hormone (thyroid hormone) but moves into the nucleus when the hormone is present.
b. recruits histone deacetylases when thyroid hormone is not bound to it AND recruits histone acetylases when thyroid hormone is bound to it. *
c. binds its hormone and then recruits hsp90 in the nucleus.

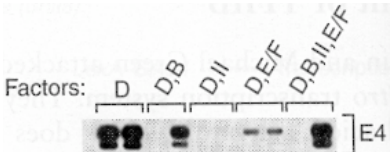
21. ***Homeodomain transcription factors have***

a. two amphipathic helices that terminate in an alpha helix rich in basic amino acids. These helices dimerize with each other on one end and bind DNA on the other end.
b. two Zn atoms each bound by six cysteine residues.
c. 60 amino acid DNA binding domains and are sometimes repressors and sometimes activators. *

22. ***The DNA binding domain of CREB belongs to the***

a. basic zipper class. *
b. repressor domain class.
c. homeodomain class.
d. hormone receptor class.

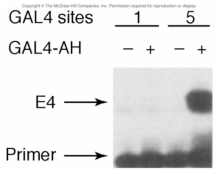




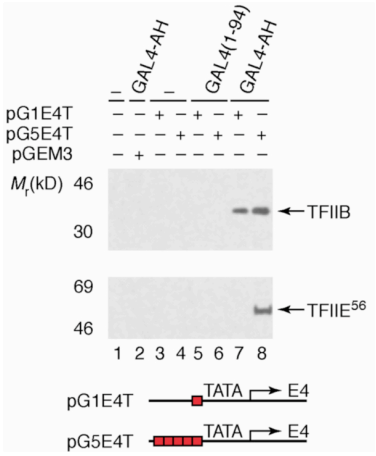
23. ***This experiment by Lin and Green (using a hybrid VP16:Gal4 transcription factor) was interpreted to mean that***

a. the acidic activation domain of this transcription factor recruits a histone deacetylase.
b. the acidic activation domain of this transcription factor directly recruits RNA polymerase II.
c. the acidic activation domain of this transcription factor helps to recruit TFIIB. *
d. the acidic activation domain of this transcription factor recruits TFIID.

Primer extension



Western blot



24. ***In this experiment, each red box represents a Gal4 binding site. This experiment indicated that***

1. a single Gal4 works as well as a five Gal4 binding sites.
2. each Gal4 binds both TFIIB and TFIIE.
3. multiple Gal4 binding sites stimulate transcription more than just one Gal4 binding site.
4. multiple Gal4 binding sites greatly increase the recruitment of a mature pre-initiation complex.

Choose the best combination.

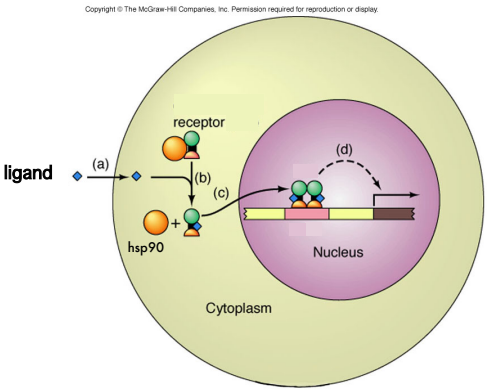
a. 1 and 2 are correct
b. 2 and 3 are correct
c. 3 and 4 are correct *
d. 4 and 1 are correct

25. ***Which statement is correct?***

a. RNA polymerase I transcribes mRNAs, RNA polymerase II transcribes tRNAs, and RNA polymerase III transcribes rRNAs.
b. RNA polymerase II transcribes mRNAs, RNA polymerase I transcribes rRNAs, and RNA polymerase III transcribes tRNAs. *
c. RNA polymerase II transcribes mRNAs, RNA polymerase I transcribes tRNAs, and RNA polymerase III transcribes rRNAs.

26. ***Conversion of RNA polymerase II from an initiation-specific form to an elongation-specific form involves***

a. phosphorylation of Gal4.
b. phosphorylation of the CTD domain of RNA polymerase II. *
c. phosphorylation of the IIb form of RNA polymerase II.
d. phosphorylation of TBP.



27. ***This diagram represents***

a. a C2H2 Zinc finger transcription factor binding its ligand.
b. a transcription factor responding to the presence of a mammalian hormone. *
c. the yeast Gal4 transcription factor responding to the presence of the sugar galactose.

28. ***In a Western Blot***

a. a pre-initiation complex is allowed to form on tethered DNA and then the protein is visualized using a peptidase.
b. a protein mixture is separated on a gel, transferred to a membrane and probed with an antibody that recognizes one specific protein. *
c. run-off transcription produces RNA that is run on a gel and visualized using a DNA probe.

Poster questions

29.

In the paper *A mediator required for activation of RNA polymerase II transcription in vitro* by Flanagan et al 1991 what data shows that mediator is required for activator function and is not just a general transcription factor?

A) When fraction a is further purified, combining it with the other fractions results only in basal transcription.
B) When both GAL4-VP16 and GCN4 are present they interfere with each other, and mediator has no effect on transcription without the activator. *
C When activator is present increasing amounts of mediator produce more transcription.

30.

In the paper *Mechanism of TATA-binding protein recruitment to a TATA-less class III promoter* by White et al 1992 the authors were studying the role of TBP in TATA-less promoters. What was the purpose of using HTNE (heat treated nuclear extract) in the experiment?

A) To provide a source of TFIIIB.
B) To remove TBP so that it could be added back and studied in a controlled manner.
C) To destroy TFIIIC so it would not bind TBP.
D) A and B *
E) A and C