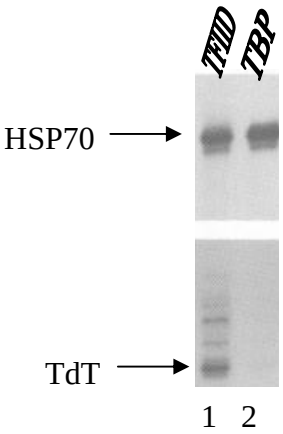
Discussion Questions Chapter 11

1. In what way are TAFI, TAFII and TAFIII proteins similar? In what way are they different?

2. Outline invitro and in vivo evidence that TAFII's are dispensable for transcription.

3. What is the evidence that TFIIB is necessary for binding of RNA Polymerase II to promoters?

4. Figures 11.10, 11.11 and 11.12 demonstrate a role for TAF's in binding core promoter elements beyond the TATA box. Figure 11.13 suggest these TAF's may be essential for initiation at TATA-less promoters. To test this suggestion, Roeder et al tested the ability of TFIID and TBP to direct transcription from TATA promoter (Hsp70) and a TATA-less promoter (TdT) which contained both an INR and DPE. They conducted invitro transcription from each promoter in extracts containing the full TFIID (lante 1) and extracts containing only the TBP (lane 2). Are their data consistent with the model presented in Figure 11.13. Explain your answer.



5. Name the TBP-containing factors involved in Pol I, Pol II, and Pol III transcription, respectively.

6. What is the role of TFIIH in initiation?

7. Pol III transcription involves an internal promoter. Describe how the transcription start site is determined with the internal promoter.

8. TFIIB is reported to interact with BRE's. Outline a DNase protection assay that could test this hypothesis. What results would support the hypothesis, what would not support it. Why is it necessary to include a "TBP only" control in the experiment.

9. Explain why mutations in the gene for TBP typically result in a loss of transcription from all classes of genes. How might TRF1 allow for an exception to this rule.

10. In class we discussed the evidence that TFIIH is the helicase that opens the transcription bubble. However your book also discusses that PIC lacking TFIIH and TFIIE generate aborted transcripts? Explain why these data are inconsistent with the idea that TFIIH is the helicase necessary for generating the transcription bubble.

11. How did Roeder demonstrate that TFIIS is only involved in elongation and not initiation?

12. Explain the relationship between TFIIS enhancement of elongation and its enhancement of proofreading.

13. Figure 11.32 Tjian determined the DNase footprint of SL1 and UBF on a RNA polymerase I promoter containing both a UPE and Core element. What did they conclude about the footprint of SL1 and UBF. How would you expect the footprint to change in the UPE was deleted from the DNA? Explain your answer.

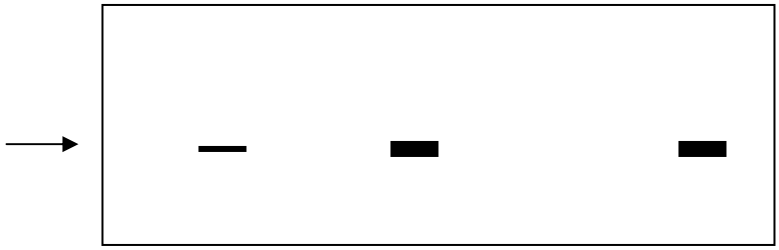
14. Outline and experiment that demonstrates that TAFII's are needed for transcriptional activation by activators such as SP1.

15. Outline experiments that demonstrate that different activators require different TAFII's.

16. Consider the following mobility shift assay. A labeled Pol II promoter DNA fragment is incubates with TFIID (called Sample 1). In another tube, the same labeled DNA fragment is incubated with TFIIB (called Sample 2). Which sample (1 or 2) will run farther or faster on the polyacrylamide gel? Why?

17. The following gel was produced using a cell extract that was immuno-depleted for TFIIE and TFIIH. All other components necessary for transcription were included. The researcher was assaying for the transcription of actin mRNA (marked with an arrow). Lane 7 is a positive control using an extract that was not immunodepleted. Lane 6, marked with an asterisk, was produced using a deletion mutant of RNA Polymerase II (a portion one of the protein complex subunits was deleted).

Lane	1	2	3	4	5	6*	7
TFIIE	-	-	+	+	+	+	
TFIIH	-	+	-	+	+	+	
ATP	+	+	+	+	-	+	



a. What would be the appropriate name for this type of experiment?

b. If TFIIE required for transcription of the actin mRNA? Which two lanes, when compared, best supports your conclusion. Explain your answer.

c. Is TFIIH required for transcription of actin mRNA? Which two lanes, when compared, best illustrate this? Explain your answer.

e. What does this experiment suggests is the role of TFIIE

d. Is transcription of actin mRNA ATP dependent? Which lane illustrates this? Howe is this consistent with the enzymatic activities of TFIIH?

e. Using what you know about the function of TFIIH, what region of RNA Polymerase II is most likely missing in the RNA Polymerase II deletion mutant used in lane 6? Explain your answer.