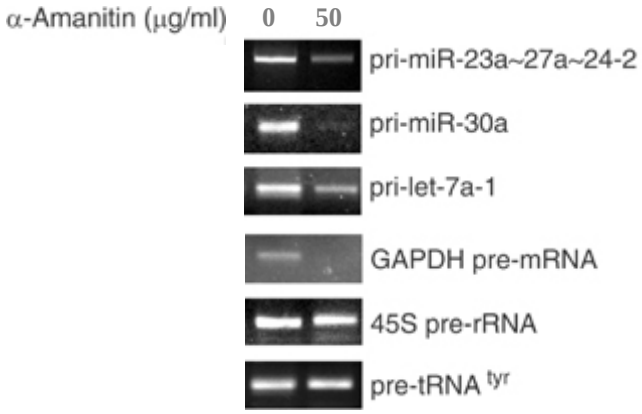
Discussion questions Chapter 10.

1. To investigate which RNA polymerase transcribes micro RNA's Lee et al (2004) treated a human cell line with alpha amanitin and then measured accumulation of various transcripts using RT PCR. They concluded that RNA polymerase II transcribed the miRNA genes. Explain how these results support this conclusion. Why did they measure the accumulation of non-miRNA's (i.e. GAPDH pre mRNA, 45S pre rRNA and pre-tRNA)?



2. Why is RNA polymerase III called "III" and not I, II or IV?

3. If I assigned you the task of purifying TFIID by epitope tagging, explain the steps that would be necessary.

4. Explain the roles of the following domains in the RNA polymerase II. Rudder, Fork Loops, Bridge Helix, Pore, Wall, Lid and the Zipper.

5. What happens to the nascent RNA chain during backtracking. What triggers backtracking?

6. Distinguish between the E site and the A site of the polymerase active site. How does the A site insure nucleotides with appropriate bases get added to the growing RNA chain?

7. What triggers movement of the bridge domain?

8. Saturation mutagenesis of a promoter region will occasionally identify a nucleotide in a positive promoter element that when mutated greatly increases transcription. What is the most likely explanation for why a mutation in a positive element would increase transcription?

9. How did Chambon demonstrate that the TATA box of the SV40 promoter determined the location of the start of transcription?

10. What is the distinction between a core promoter elements and a proximal promoter element?

11. Explain how deletion of Inr elements in TATA containing promoters suggests that the Inr plays a role in determining the location of the start of transcription.

13. Explain the evidence that the DPE only works in conjuction with a Inr.

14. Suppose you identified a positive promoter element 100bp from the start of transcription. Describe an experiment that would allow you to investigate whether the element had the properties of a classic “enhancer” (i.e. distance and orientation independence).

15. Discuss the role of TATA box binding and transcription by Pol III genes.