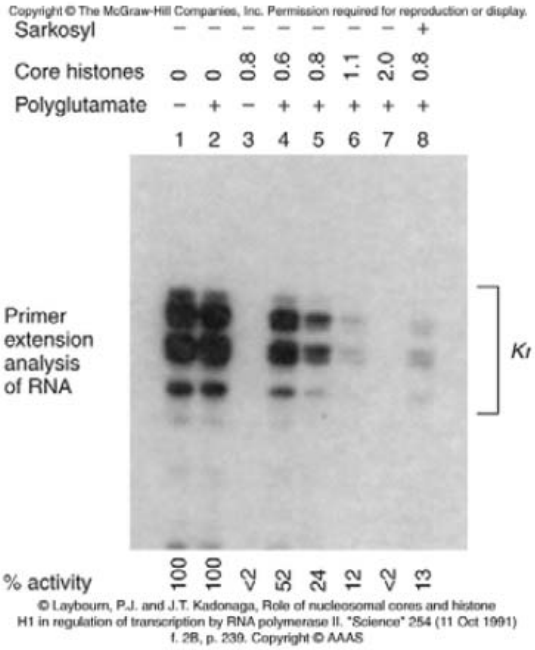
1. In Don Browns experiment of Fig 13.12, what was “accidentally” derepressed chromatin. Why did he conduct the experiments outlined in Figure 13.12b lanes 2 and 3?

2. In the figure below, what is the significance of lane 3? Why is lane 5 believed to best represent what goes no in the cell? Why is there so much less transcription in lane 7 compared to lane 5?



3. In corn the nuclear gene for Rubisco is only expressed in green leaves and not in other tissues including roots. A study of general DNase sensitivity was used to compare the general chromatin structure of this gene. Nuclei were isolated from both tissues and treated with varying amounts of DNase. The DNA was isolated from the nuclei, digested with restriction enzymes that cut sites flanking the Rubisco gene, the digested DNA was separated by gel electrophoresis, blotted to a filter and hybridized to a probe for the Rubisco gene. Diagram the results that would support a model of open chromatin structure for actively transcribed genes and closed chromatin structure for transcriptionally inactive genes.

4. What is a corepressor? Explain how co-repressors reduce expression of specific genes. Describe two mechanisms that co-repressors can use to reduce transcription.

5. Outline experimental evidence that acetylated chromatin has a more open structure and unacetylated chromatin.

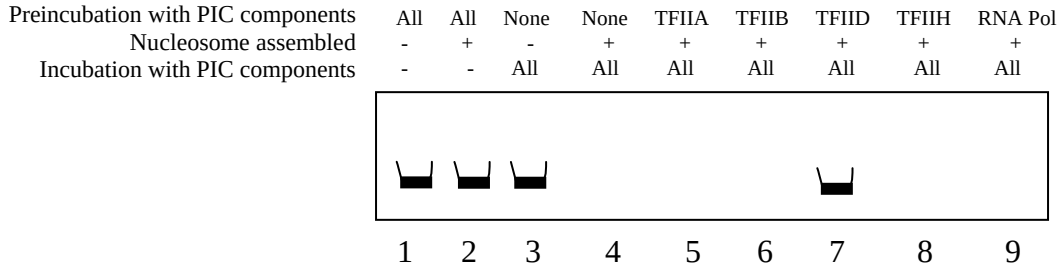
6. Outline how you might determine if TFIID binds the TATA box of the Rubisco gene of corn in root nuclei in vivo.

7. Explain why the chromodomain of the HP1 protein is important to spread of chromatin repression by histone methylation.

8. $TAF_{II}250$ has a bromodomain. How might this bromodomain facilitate "regulated" transcription of genes? Be sure to explain its role in regulation.

9. The nucleosome core is described as having a tripartite structure. What are the three components that make it "tri"partite?

10. If DNA is incubated with PIC components (RNA Polymerase II and GTF) before nucleosomes are assembled, transcription can occur (lane 2). However if nucleosomes are assembled before RNA Polymerase II and GTF are added, transcription is inhibited (lane 4). This suggests that components of the PIC can keep promoter region in a transcription competent state. The investigate which component was critical to keep promoter in transcription competent state during nucleosome assembly, the DNA template was preincubated with a single factor of PIC. After nucleosomes were assembled all the factors were added to the DNA and transcription was measured (lanes 5-9). What conclusion would you draw from these results?



11. Galactose metabolism genes are only expressed when cells are grown on galactose. Expression of these genes is dependent on GAL4. Design a CHIP assay to determine if GAL4 only binds the UAS of Galactose metabolism genes when galactose is present or if it is always bound to the UAS. Discuss the possible results.

12. The chromatin chaperone FACT (SP16 + SSRP1) bind to the phosphorylated CTD of RNA Polymerase II. What is the significance of this interaction?

13. While most CHIP assays are similar, they often differ in which antibodies are used and which PCR primers are used. How does the choice of antibody affect the CHIP assay? How does the choice of PCR primers affect the CHIP assay?

14. The SAGA HAT complex contains a Bromodomain as well as a HAT domain. Explain the role of the Bromodomain in the exchange of co-activators at the activator. Discuss the role of the bromodomain in the spread of "active" chromatin through a gene. Explain how this spread of "active" chromatin partially explains how activators can work at a distance.

15. The figure below is Kadonaga's experiment to study the role of H1 histones in activation.

a. Site two lanes which most clearly demonstrate that H1 has a repressive effect on transcription.
b. Lanes 5 and 6 most clearly illustrates that activators can function on chromatin, even chromatin containing H1. What is the argument that activitors have a "anti-repression" activity with regard to H1 repression and that the activation in these lanes is not just due to activators interaction with the PIC?