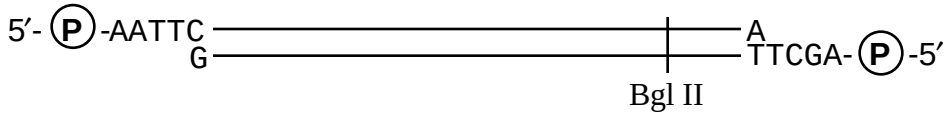
**Biochemistry 674: Nucleic Acids** **Your Name:** _____

**Exam II, November 19, 1996** **Prof. Jason Kahn**

There are five questions on this exam, worth 20 points each (really). You do not need a calculator. Answer the easy questions first, guess if you don't know, and give clear and concise answers. You have 85 minutes for this exam.

## 1. Methods

a. (8) Describe two ways to label the ends of the Eco R I - Hind III restriction fragment shown below, using either $\gamma$-$^{32}$P-rATP or $\alpha$-$^{32}$P-dATP. List the enzymes and substrates needed and sketch the final products of each method at the Eco R I (left) end, indicating where the radiolabel is by a *. The (P) indicates a 5′ phosphate.




(4) In order to do footprinting analysis, we need a molecule with only one end labeled. Why? How could such a molecule be obtained from the material generated above? How could this have been done in one step using a different source of radiolabel?

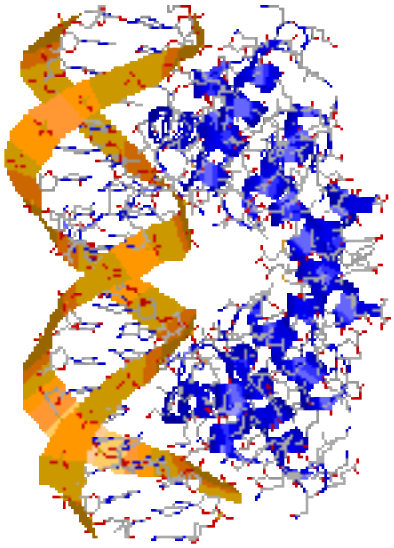
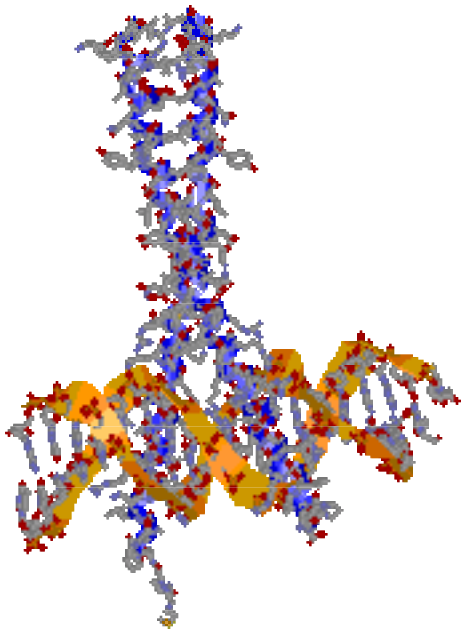
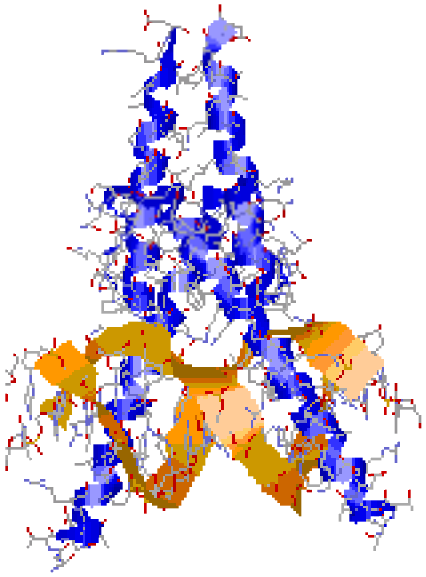
b. (8) Name two advantages of nitrocellulose filter binding over gel shift analysis in analyzing protein-DNA interaction, and two advantages of the gel shift analysis. Hints: speed, assembly and stoichiometry, protein concentration, sensitivity.

**2. Protein-nucleic acid interaction**

a. (5) Arginine is commonly found recognizing guanosine in protein-DNA complexes. Draw the structure of the guanidinium group of arginine hydrogen bonding to the Hoogsteen face of G. Why else is arginine commonly found in nucleic acid binding domains?

b. (5) On the pictures of the leucine zipper (GCN4), helix-loop-helix (MyoD), and helix-turn-helix (lambda repressor) cocrystal structures below, label which complex is which, circle the DNA binding domains, and draw arrows indicating the dimerization domains.

Also, name another class of DNA binding motif:_____

| Name:_____ | Name:_____ | Name:_____ |
|---|---|---|
|  |  |  |

c. (5) Why is non-specific DNA binding typically more salt-dependent than sequence-specific binding? What is the usual driving force for non-specific binding?

d. (5) How is RNA recognition in the tat-TAR system fundamentally different from "typical" DNA recognition, e.g. by HTH proteins?

## 3. DNA replication

a. (8) Sketch the incorporation of a deoxynucleoside triphosphate at a template-primer (just the covalent chemistry, nothing about the active site). Give a biochemical rationale for the obligate 5′→ 3′ direction of DNA and RNA synthesis, given that we have only 5′ triphosphates available.

b. (4) Why might the use of RNA primers as opposed to DNA have been evolved/maintained in chromosome replication by DNA polymerase III? What happens to the primers during replication?

c. (4) Sketch the chromosome of *E. coli* during rapid growth, at a time just before the completion of chromosome duplication, assuming a 20 minute doubling time and a 40 minute chromosome duplication time (*i.e.* the picture I drew in lecture).

d. (4) What are the two features of the 3′→ 5′ exonuclease activity of Klenow fragment which contribute to the exo's enhancement of polymerase fidelity?

**4. Prokaryotic Transcription**

a. (5) Name the four steps of the prokaryotic transcription cycle. Which two are studied using the abortive initiation assay? Which two are studied with "walking" methods? Where does promoter escape fit in?

b. (7) Which step of the cycle is affected by NtrC protein? What is its target? How does NtrC find its target? Briefly describe one experiment in support of the target location mechanism you describe.

c. (4) How is the α subunit of *E. coli* RNA polymerase proposed to mediate transcription activation?

d. (4) What are two functions of σ subunits of *E. coli* RNA polymerase? Under what circumstances (in general) do we see the use of alternative σ factors?

**5. Eukaryotic transcription and replication**

a. (7) How does chromatin generally act in eukaryotic transcription? Give a counterexample, mentioning what is special about the nucleosome in question. Write one word describing how the eukaryotic transcription machinery deals with chromatin.

b. (4) What comprises TFIID (no numerals needed in answer)? What does it do?

c. (4) What are possible functions for topoisomerase II in matrix attachment regions?

d. (5) What problem does the combination of RNA primers and 5′→ 3′ replication described above create for replication of linear chromosomes? How is this problem solved in eukaryotes?

| Question | Score |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| Total | |