Biochemistry 674 **Your Name:** _____

Nucleic Acids **Prof. Jason Kahn**

Exam II (100 points total) **November 13, 2006**

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. There is a extra space on the last page if you need it.

You do not need a calculator for this exam, and no other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the end of the examination time , please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. (6 pts) Differentially methylated histone H3's are distributed vectorially along a gene as shown in the figure at the right. (The details of which methylation state is where are not important.) The results were obtained using ChIP. Why is the time used for shearing the chromatin an important aspect of obtaining the above result, i.e. how could one go wrong if the shearing was done for too short a time?

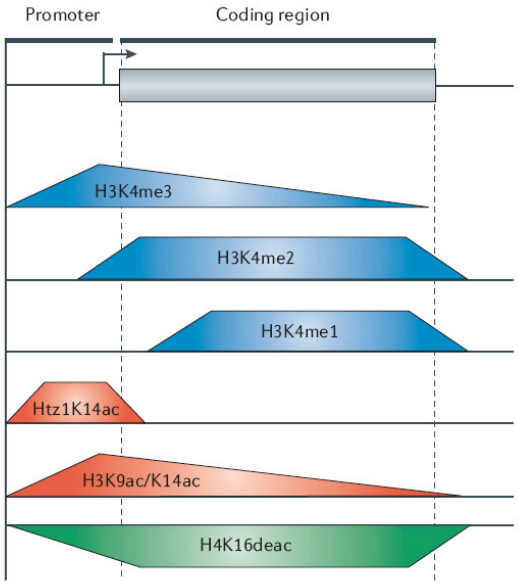

Figure 3 | **Gradients of histone methylation and acetylation in active genes.** A schematic representation of an active euchromatic gene is shown, with the promoter and coding regions indicated. The extent of modification at H3K4, H3K9, H3K14, H4K16 and Htz1K14 are shown. The level of modification is depicted by the height of the coloured shape. H3K4me3 is most enriched at the 5′ end of active genes, whereas H3K4me2 and H3K4me1 are found in increasing amounts at the 3′ end of the coding region. Acetylation of Htz1K14 is found at promoters, whereas acetylation detected by antibodies to H3 diacetylated at K9 and K14 peaks at the 5′ end of genes. H4K16 is deacetylated at active genes and promoters.

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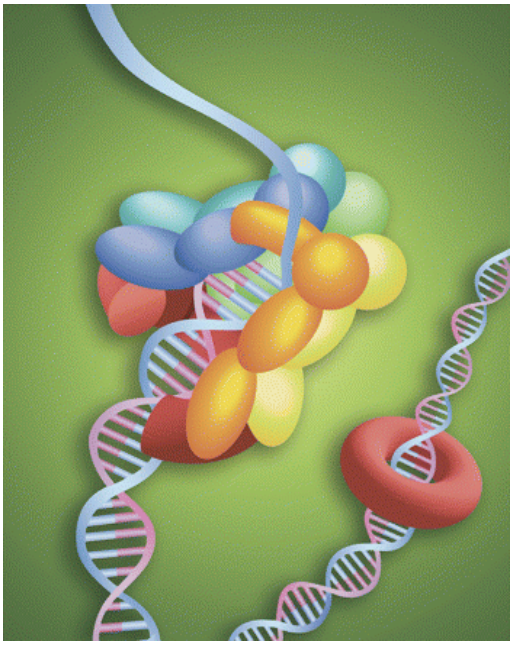
2. (6 pts) The sketch at the right shows an artist's view of a eukaryotic clamp loader•ATP•sliding clamp•DNA structure, from the cover of *JBC* this week. The top collar of the clamp loader requires the DNA to bend dramatically upon exiting the complex. Why does this lead to improved binding to a ssDNA-dsDNA junction relative to ds DNA? Why does this binding preference make mechanistic sense in terms of the function of the clamp loader?



3. (4 pts) We have emphasized that AAA+ proteins like the $\gamma_3\delta\delta'$ clamp loader must hydrolyze ATP in order to work effectively. However, it has been observed that the δ subunit alone binds preferentially to the open β clamp and therefore might catalyze unloading without using ATP. So, what is the essential role of the ATPase of the complete clamp loader?

4. (6 pts) In general, why do we eschew (avoid) arguments based on being economical with ATP when we talk about how nucleic acid transactions work? What happened to the primordial bacteria that replicated their genomes using 15 % less ATP than their competitors but had overall DNA replication error rates of $1/10^{-4}$?

Score for the page_____

5. (10 pts) Sketch two ways to make singly end-labeled DNA starting with a restriction fragment with 5′ phosphate overhangs.

6. (9 pts) What mode of protein-nucleic acid recognition is described by the "H bonding matrix?" Briefly describe the idea of the H-bonding matrix.

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7. (6 pts) Many DNA binding proteins employ an α helix in the major groove to recognize a sequence, but a single helix is never the sole contact. Why not? Why don't RNA binding proteins recognize fully double-stranded RNA using an α helix in the major groove?

8. (4 pts) Which of the following DBDs can be identified by looking for parallel α helices 34 Å apart (circle one)? Zinc finger, bZIP protein, helix-turn helix. Bacterial homodimers of this type usually bind sequences with a type of symmetry; name it:

9. (4 pts) Which of the following uses a β sheet for DNA recognition (circle it)? TBP, GCN4, the Max bHLH protein. What mode of recognition provides its specificity?

10. (10 pts) What is the equation for fractional saturation as a function of protein concentration, assuming that $[P]_{total} = [P]_{free}$. Experimentally, what do you have to do to ensure that this assumption is correct? Why can that sometimes be a challenge if the protein-nucleic interaction has a very small dissociation constant?

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11. (3 pts) We discussed the analogy of assembling a genome being like assembling a novel from different snippets of text clipped from many copies of the novel (for which I am indebted to a talk by Pavel Pevzner). Using the novel-assembling analogy, what would correspond in real life to the problem that before clipping up the books an official censor removed the subversive page 27 from each one?

12. (6 pts) Give two reasons for sequencing every base several times (on average) when doing WGS (whole genome sequencing).

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13. (8 pts) There are at least two reasons that a mutant DNA polymerase capable of rapid addition to a mismatch would exhibit decreased fidelity even if the base selection step had the same fidelity as wild type. One reason is that the base added to a mismatched terminus might also be mismatched – why? What is the other reason for the expected low fidelity, based on the ideas of kinetic proofreading?

14. (8 pts) Sketch the mechanism of the 3′ to 5′ exonuclease mechanism for a DNA polymerase.

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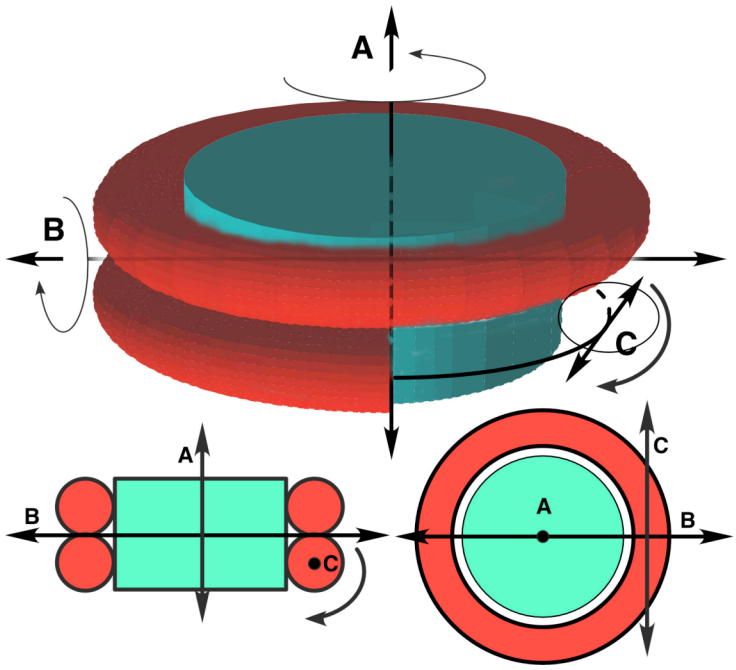
15. (6 pts) "Nucleosome rotational positioning." In the crude sketch below, which axis is the axis to which the rotation of the phrase refers? Which axis could be the dyad axis of the nucleosome? Is axis "A" a symmetry axis for the actual nucleosome structure?



16. (4 pts) Why can't a nucleosome slide 5 bp along the DNA without changing rotational positioning?

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