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?

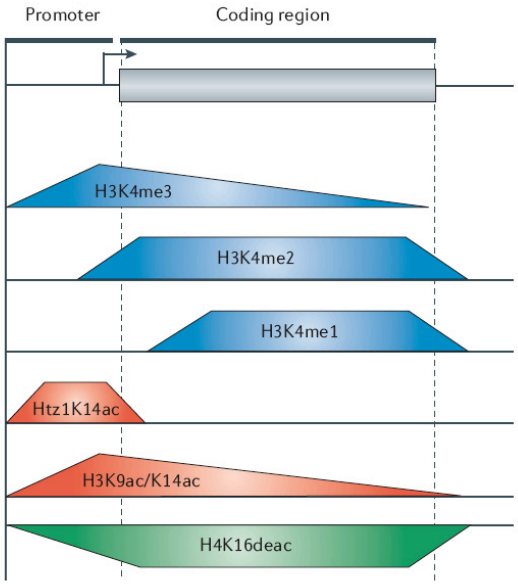
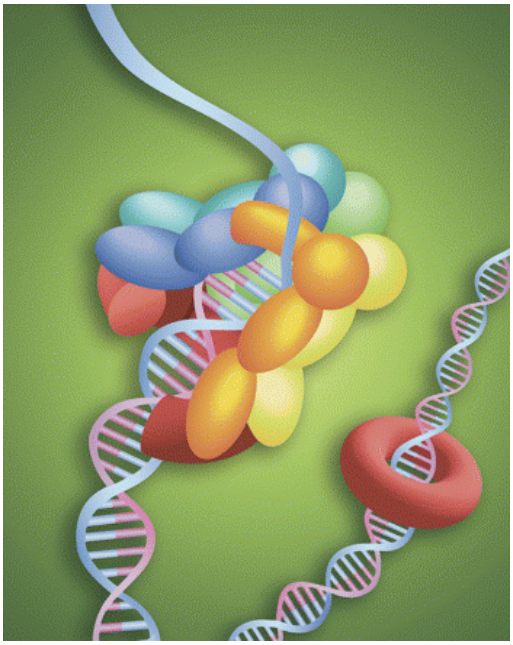



Figure 3 | **Gradients of histone methylation and acetylation in active genes.** A schematic representation of an active euchromatic gene is shown, with the promoter and coding regions indicated. The extent of modification at H3K4, H3K9, H3K14, H4K16 and Htz1K14 are shown. The level of modification is 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.

The experiment would be to IP with antibodies to the modified histones (H3K4me, H3K4me2, H3K4me3). Analysis of the distribution along a gene requires sufficient resolution to distinguish the promoter from the proximal coding region and then to see differences along the coding region. If the chromatin is sheared only lightly, the fragments will be so large that the whole area could light up as a result of IPing a protein anywhere on it. One would miss the whole gradient.
+3 for the idea of how the experiment would be done (implicitly is OK)
+3 for connecting the time of sonication/shearing to ChIP resolution

Score for the page_____

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 dsDNA is much stiffer than ssDNA, therefore the bending free energy will be much less for a ss-ds junction than a pure duplex.
+3 This allows the clamp loader to be selective for the place where the sliding clamp is needed, the site where the core polymerase will start extending. Otherwise it could end up on the lagging strand, ahead of the helicase, etc.

[This does bring up the interesting question of how the clamp loader can unload the clamp from duplex DNA. The prediction would be that unloading would work much better on nicked or heteroduplex DNA.

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?

The ATPase is needed for determining unidirectionality, speed, and coordination. ATP binding is coupled to clamp binding, ATPase is stimulated by clamp and DNA binding, and the clamp is released by the ADP-bound. This leads to rapid binding, loading, and release, and minimizes the unloading of the most-recently loaded clamp.
+2 for the idea that ATPase allows for two states, one that binds the clamp with high affinity and one that releases it.
+2 for the idea that this is important for cycling the clamp-beta interaction.

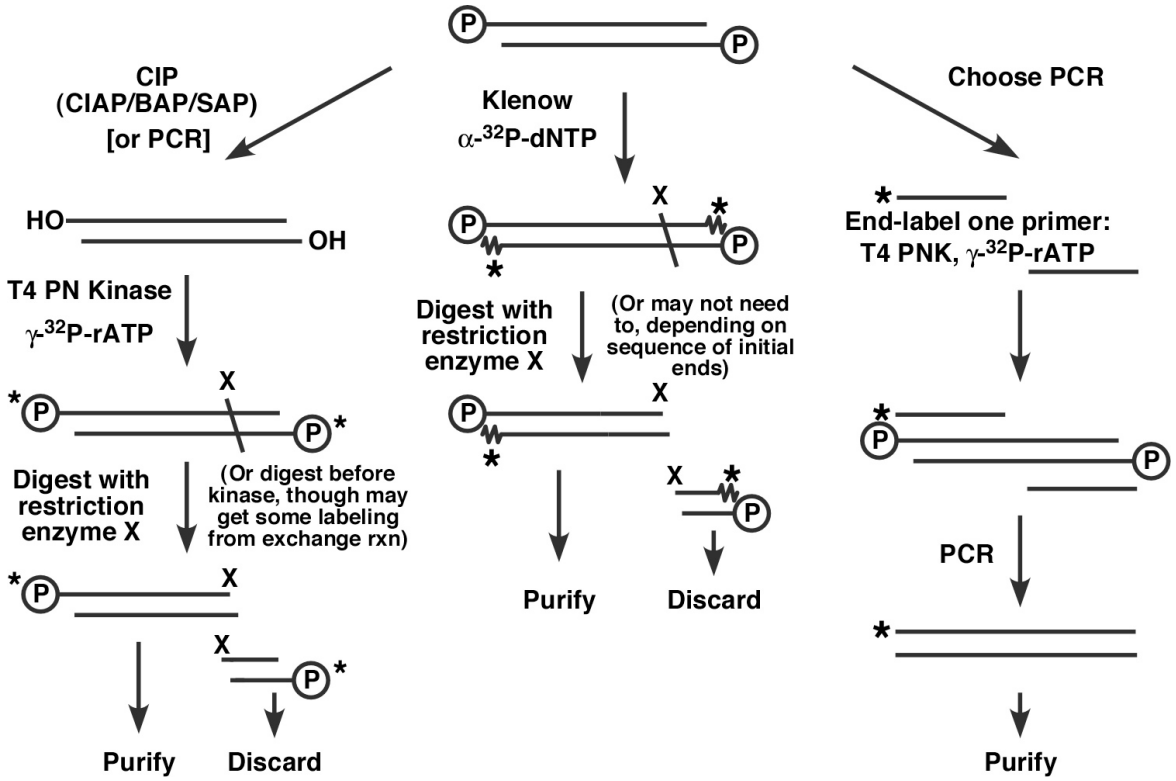
4. (6 pts) In general, why do we eschew (avoid) arguments based on being economical with ATP when we talk about how nucleic acid transactions work? What happened to the primordial bacteria that replicated their genomes using 15 % less ATP than their competitors but had overall DNA replication error rates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

+3 We avoid arguments based on economy because for nucleic acid transactions they generally don't work! Kinetics and fidelity are much more important: speed and precision are expensive but worth it.
+3 Primordial creatures that copied their genomes with low fidelity were unfit because they ran into the error catastrophe: they accumulated mutations so rapidly they could not survive. Only the small viral genomes can survive, because there is a reasonable chance that progeny virus will have no or few mutations, and the benefit of rapid adaptation to host defenses balances the likelihood that many progeny viruses will be defective.

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.

Several possibilities. For each of two, +2 for just naming it and +3 for a correct sketch. You need to make it clear where the label comes from.




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.

+3 Direct Readout

+3 The surface accessible to DNA-binding proteins is a stripe of phosphate, sugars, and the edges of the base pairs (the major and minor grooves).

+3 The four W-C base pairs are all distinguishable by the H-bonding donors and acceptors available in the major groove.

Score for the page_____

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?

+3 Binding affinity is vaguely proportional to the surface area of the interface. The contact area for one α helix is too small to provide enough binding affinity or specificity

—OR—

+2 An α helix is usually not stably folded in solution, and the binding affinity is too low to drive coupled folding (if this were true, one might expect a folded protein to present just the helix, which doesn't happen).

and

+3 The major groove of dsRNA is too narrow and deep to admit an α helix

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:

+2 Helix-turn-helix; +2 Palindrome or inverted repeat

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?

+2 TBP, +2 deformability or induced fit, [+1 for indirect readout]

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?

+3 $\Theta = P_T/(P_T + K_{diss})$

+3 To have $[P]_{total} = [P]_{free}$ we must have negligible [P•D] relative to $[P]_{total}$. This is equivalent to requiring $[DNA] << K_{diss}$.

+2 The challenge is that we need to work with very low [DNA].

+2 This requires very high specific activity or large sample volume.

Score for the page_____

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?

+3 This would correspond to “non-clonable” DNA either due to unstable DNA structure or a fragment that makes a toxic protein. The end result is a gap in the final sequence.

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

+3 The genome is assembled by overlapping sequence reads to make contigs. Anything that is an overlap must have been read at least twice! In order to ensure a high probability that every base near the end of a read will be overlapped, the average base needs to be sequenced several times.

+3 Sequencing more than once increases confidence that the sequence is correct.

+3 Multiple overlappinf assemblies reduces the chance of overcollapsing repeat sequences.

Score for the page_____

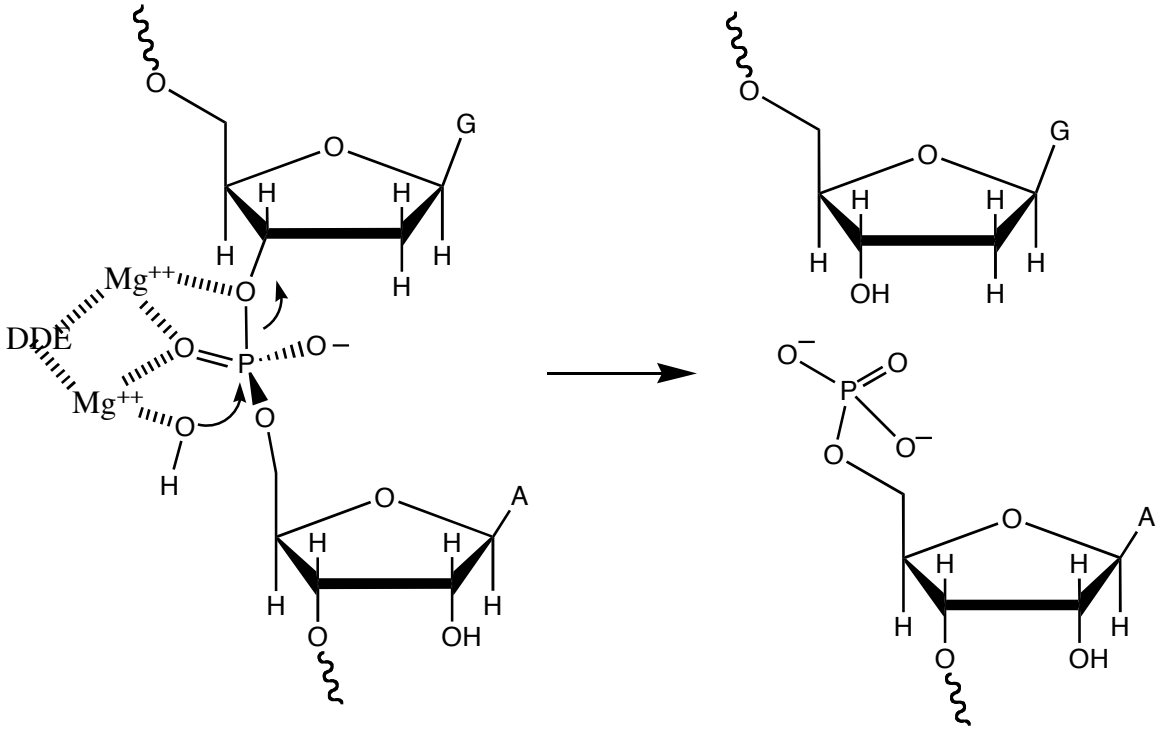
13. (8 pts) There are at least two reasons that a mutant DNA polymerase capable of rapid addition to a mismatch would exhibit decreased fidelity even if the base selection step had the same fidelity as wild type. One reason is that the base added to a mismatched terminus might also be mismatched – why?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

+2 The rate-limiting conformational change during polymerization is the closure of an α helix over the incoming dNTP.
+2 a mismatched primer terminus would disrupt close approach of this helix, making it more difficult to distinguish whether the next base is correct or incorrect.

+2 The essence of kinetic proofreading is that a low-specificity 3'->5' exonuclease has time to act on the 3' end because it is extended very slowly by the 5'->3' polymerization activity.
+2 Rapid extension of a mismatched terminus would redirect kinetic partitioning toward extension rather than removal, thereby sealing in the mismatch.

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

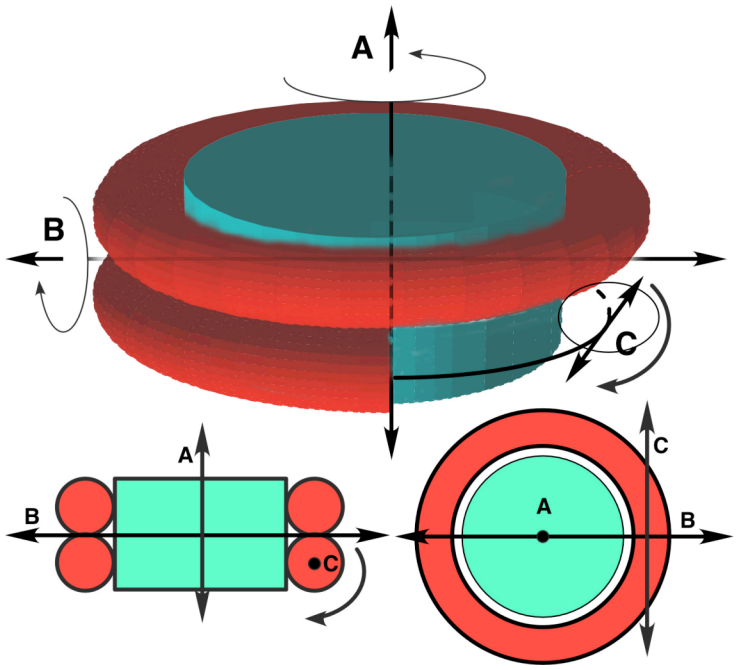
+2 for phosphodiester backbone
+2 for attack by water or $OH^-$ at the phosphorous
+1 for reasonable in-line geometry, arrow pushing
+1 for two metal ions
+2 for 5'dNMP and 3'-OH products




Score for the page_____

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?

+2 Each:
Axis C: rotation about the DNA helix axis
Axis B could be the dyad
Axis A is not a symmetry axis




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

There's a ramp of positive charge on the histone octomer to which the DNA binds. Shifting the DNA by 5 bp would change the position of all of the backbone atoms (+2) with respect to the surface of the octamer, whereas shifting 10(.2) bp would retain the same "inside" and "outside." A 5 bp shift would be accompanied by a half-turn of rotation about Axis C to bring the DNA backbone into the correct register again, but with a new rotational positioning (+2).

| Page | Score |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |

Score for the page_____