MCB 110
Spring 2003

Midterm
February 24, 2003

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You are given 5 questions below. Answer only 4 of them. If you answer all 5, only the first 4 answers will be graded.

1. We have seen a number of examples of a nucleophilic attack in which the reaction was highly energetically favorable. For example, the incorporation of deoxyribonucleotides by a DNA polymerase. List five (5) distinct examples of a nucleophilic attack in which the free energy change (ΔG) is close to zero such that the equilibrium constant for the reaction is near one. In these cases, the reaction is energetically neutral, or nearly so. In each case, state briefly in chemical terms why ΔG is near zero and what is the physiological advantage of a ΔG near zero.

1. Formation of aa-AMP by tRNA synthetase
   $\Delta G \approx 0$ because trading one anhydride (pyro) for another (phosphoric and carboxylic acids). Need to transfer aa to tRNA and therefore need to keep high energy bond.

2. Formation of aa-tRNA from aa-AMP by tRNA synthetase
   $\Delta G \approx 0$ because ester to tRNA has 2'OH neighbor and therefore has high energy. Need high energy bond so that peptide bond formation is favorable.

3. Formation of E-DNA intermediate in site-specific recombination and reformation of phosphodiester bond in product.
   $\Delta G \approx 0$ because they are ester exchange reactions between comparable esters. Need to conserve energey since no ATP hydrolysis involved in site-specific recombination.

4. Type-1 topoisomerases. Like answer 3 above.

5. Formation of DNA adenylate by DNA ligase from ATP
   Exchanging a phosphoanhydride for a high-energy phosphoramidate. Need to conserve the energy for the downhill step of phosphodiester bond formation.

Other similar examples in transposition.

2. Compare and contrast the mechanisms for maintaining the fidelity of DNA replication and protein synthesis. Give the steps involved in each where errors can occur and how they are corrected.

DNA Replication (3 steps):

D1. Free energy difference between Watson-Crick and non-Watson Crick pair of dNTP to template on the DNA polymerase. Error rate ~ 10^{-5}.

D2. Editing 3' exonuclease that is single strand specific. Preferentially removes mismatched nucleotides that has just been incorporated. Error rate ~ 10^{-2}.

D3. Post replicative mismatch repair.
Digest with single strand exonuclease from a nick in the newly replicated strand to the mismatch and fill in with pol III holoenzyme. The nick in eubacteria is at unmethylated A in GATC; probably Okazaki fragments in other organism. Error rate ~ 10^{-2}. No analogue in protein synthesis.

Protein Synthesis (4 steps):

P1. Free energy difference between right and wrong amino acid binding to aa-tRNA synthetase. Analogue (sort of) of D1, but not as good fidelity. Error rate ~ 10^{-2} to 10^{-5}.

P2. Hydrolysis of misacylated tRNA for some synthetase where amino acids are similar (e.g., ileu and val). Direct analogue of D2. A proofreading of newly synthesized product via hydrolysis. Error rate ~ 10^{-2}.

P3. Dissociation of mispaired aa-tRNA•EfTu to mRNA on ribosome. Analogue (sort of) of D1 in that base pairing accuracy is being measured.

P4. Hydrolysis of mispaired proteins. No analogue in DNA replication. Cannot chew up your DNA, but have lots of copies of proteins and can make more.

3. Repetition of sub-gene-sized DNA sequences is important in many cellular processes. Name five (5) distinct examples of such repetitions in DNA sequences. Make sure that the examples are different. For example, you cannot say that Tn1, Tn3, Tn5, Tn7, and Tn10 cause a direct repeat of a short sequence in the target DNA that flanks the transposon and count that as five separate examples. (You also cannot use target sequence repeats around transposons as an example now). Indicate whether the repeat is direct, inverse, or can be either. In one sentence for each example, indicate the function of the repeat sequence. Where possible, state why the function would be different or impossible if the repeat had a different orientation or if there was a single copy of the sequence.

Extra Credit: What is the probable consequence for the secondary structure of an mRNA encoded by a gene containing an inverted repeat? Hairpin

1. Direct repetition of sites recognized by the resolvase class of site-specific recombination reactions, e.g., Tn3 resolvase. The repetition allows a single binding protein to form a synaptic complex via quaternary interactions. The result of recombination is deletion.

2. Inverted repetition of sites recognized by the invertase class of site-specific recombination reactions, e.g., Gin. The repetition has the economy stated in 1 above, even though an accessory protein (Fis) is needed for Gin. The result is inverstion.

3. GATC sequences are the site for the DAM methylase in bacteria. By having lots (~every kb) in the genome, it is likely that a hemimethylated site will be present post-replication near any mismatch and activate post-replication repair. The trick, though, is that GATC is a palindrome and thus it is meaningless to ask if its repeats are direct or inverse.

4. Inverted repeats allow for equivalent interactions with a binding protein–e.g., transposase acting on the inverted repeats at the ends of transposon.

5. OriC has two sets of repeats:

   9-mers: direct repeats to which DnaA binds, forms large protein-DNA complex recognized by DnaB and DnaC.

   13-mers: AT-rick sequences for opening DNA. Both direct and inverted repeats.

6. A restriction enyme site is usually a palindrome. It may occur once, more than once, or not at all in a given genome. Since it has 2-fold symmetry, it is undefined whether repeats are direct or inverted.

7. Direct repeats at ends of some transposons, e.g., Tn10.

8. Inverted repeat recognition sequence. Doubles length of DNA recognized.

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4. Consider a bifunctional alkylating agent of the general structure $Cl(CH_2)_nCl$ that attacks DNA. This agent can have the following consequences:

(A) It can add to a base, B, to give the product
$Cl(CH_2)_nCl$ -B. This is a monoadduct. In cases (B)-(E), a diadduct is formed.

(B) It can add to two bases in the same strand of the double helix, B_{W1} and B_{W2}, to give
B_{W1}-$(CH_2)_n$-B_{W2}

(C) It can add to bases in the complementary strands of the double helix, B_W and B_C, to give
B_W-$(CH_2)_n$-B_C

(D) It can add to bases on different chromosomes in random positions, B_I and B_{II}, to give
B_I-$(CH_2)_n$-B_{II}

(E) It can add to the same base at the exact same positions in homologous chromosome, B_M and B_D, to give
B_M-$(CH_2)_n$-B_D

In each case, indicate which repair processes would likely be important in repair of the damage. Give your reasoning.

(A) NER. Bulky adduct and effects only one strand of duplex.

(B) NER. Bulky adduct and effects only one strand of duplex. If n is longer than the length of excised DNA, then need two successive NERs and intermediate is lesion in (A).

(C) DSBR either by general recombination or NHEJ.

(D) 2 NERs with intermediate as in (A). DSBR is a much less likely alternative as one strand is still intact.

(E) 2 NERS with intermediate as in (A). If they say DSBR, it must involve NHEJ; but much less likely.

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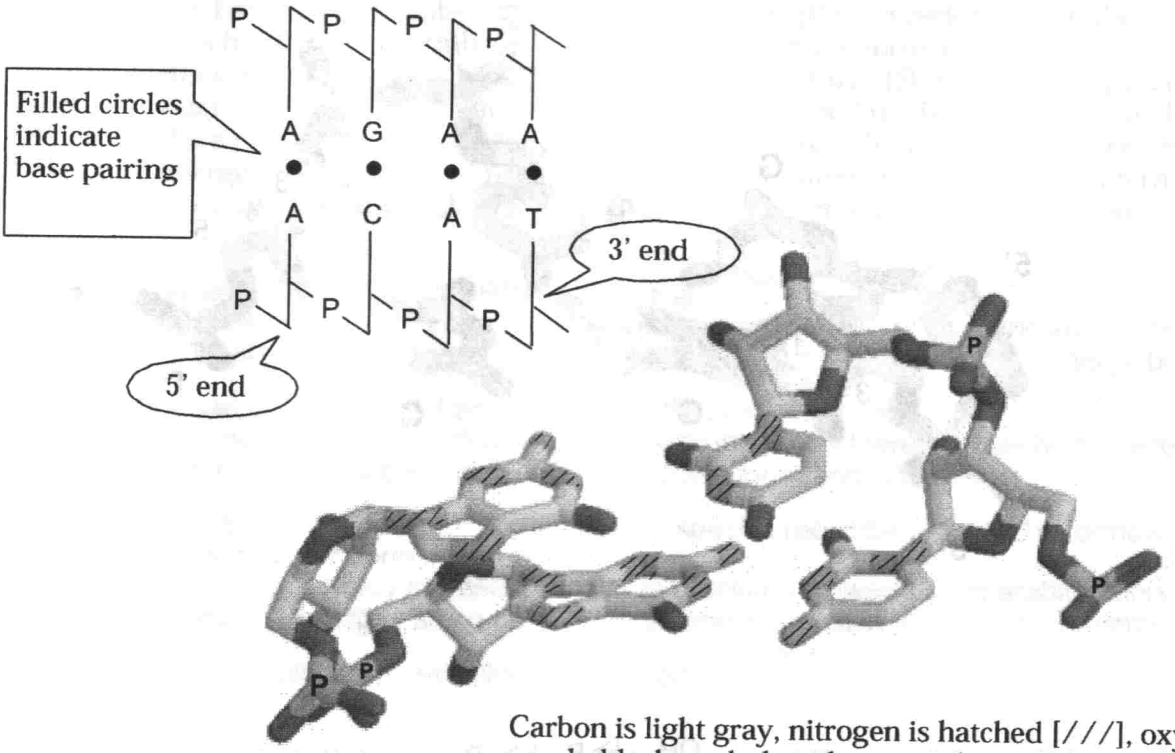
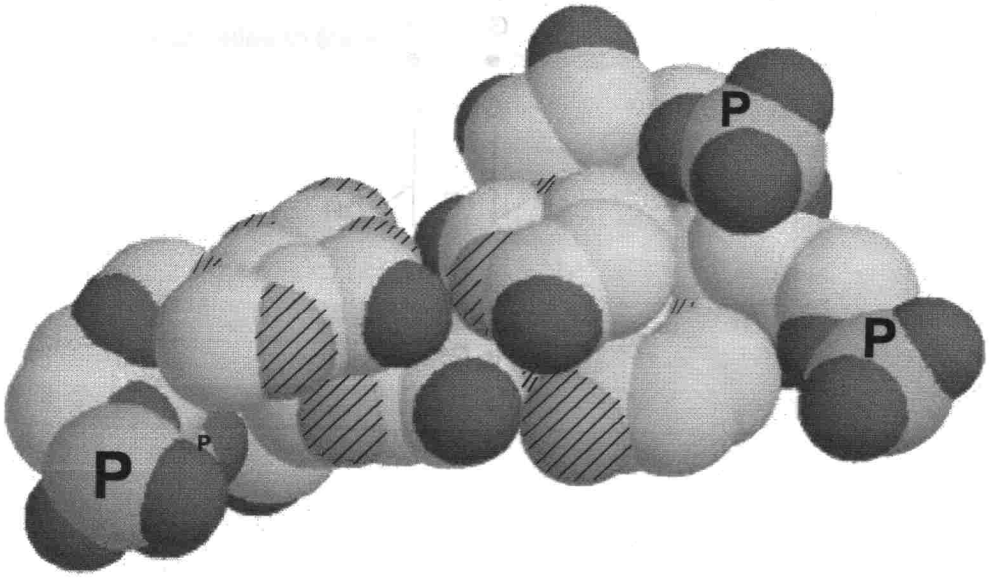
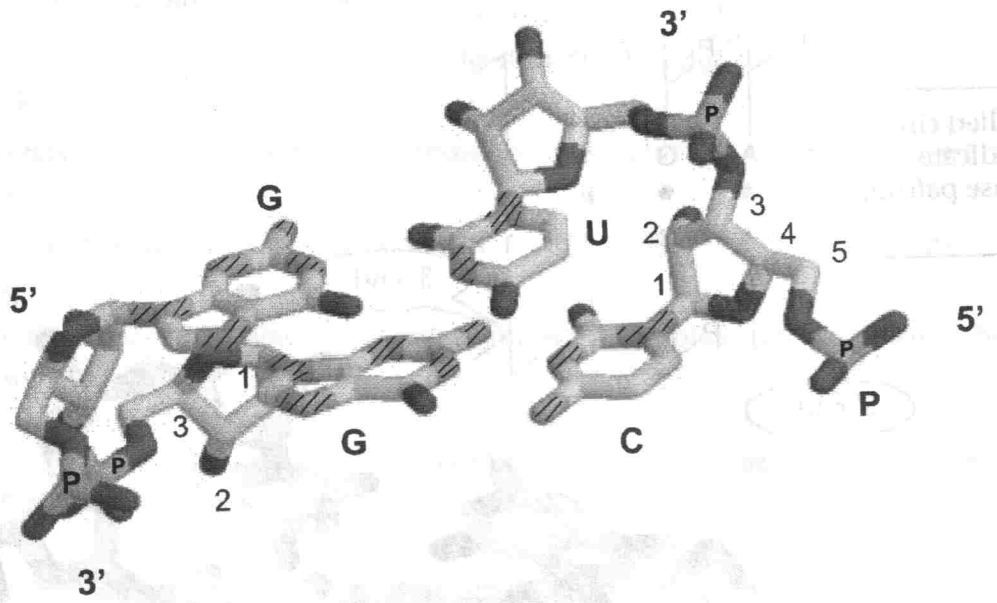
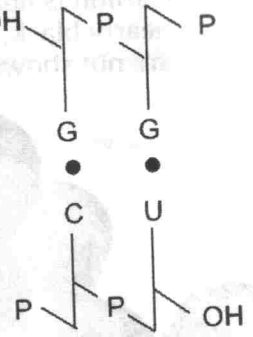
5. Shown below is a single view of a portion of a nucleic acid molecule, but in stick figure and space filling formats. H's are not idicated. Write out the nucleotide sequence and indicate polarity and base pairing. Is it RNA or DNA? Just use a shorthand for nucleotide sequences; e.g., (and this is a completely wrong answer):



Carbon is light gray, nitrogen is hatched [///], oxygen is nearly black, and phosphorous is has a "P" on it (hydrogens are not shown).







It is RNA.