Chemistry 135, Second Exam
October 12, 2018
Please only turn this page once instructed to do so by the instructor.

This exam will be worth 15% of your overall grade. Please read all of the instructions/questions carefully and answer the question in the space provided or indicated. There should **12** total pages containing **7** multi-part questions. Be sure to transfer any answers you wish to receive credit for to the space provided. No calculators, phones, electronic devices, etc. may be used during this exam. Good luck!

Questions

Question	Points	Question #	Points
1	20	4	18
2	20	5	31
3	34	6	17
		7	26
	Total	166	

Remember that whenever you take an exam, you are really taking *two* tests. The first is a test of your knowledge from the class. The second, and more important, is a test of integrity: that the answers you put down represent your answers and not someone else's. Please make sure to pass the more important test!

You will not need any calculators, phones, electronic devices, headphones, etc. to complete this exam (indeed, they will slow you down), so please make sure these are put away.

1. Please carefully evaluate the following statements. If the statement is correct, please mark it as "True." If the statement is false, please provide the correction(s) that renders the statement true. (4 points each)

For example:
The chemical biology program at Stanford is the best!
This would be marked as "FALSE" and could be corrected in the following way:

The chemical biology program at ~~Stanford~~ *Cal* is the best!

a) **FALSE** ~~RNA~~ **DNA** polymerase has a proof-reading mechanism based on ~~5' to 3'~~ **3' to 5'** exonuclease, whereas ~~DNA~~ **RNA** polymerases do not possess a proof-reading mechanism.

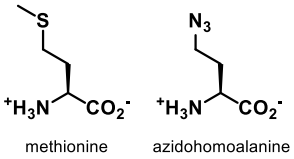
b) **FALSE** The development of DNA sequencing or so-called "Sanger sequencing", relies upon the observation that small quantities of ~~dNTPS~~ **ddNTPs or dideoxyNTPs** could be incorporated to randomly terminate the polymerization of DNA, allowing identification of the DNA sequence.

c) **FALSE** The canonical form of DNA, determined by Watson and Crick (with help from Franklin) is double helical, ~~left~~**right**-handed helix, otherwise known as "B" form DNA.

d) **FALSE** The increased sensitivity of ~~DNA~~ **RNA** to ~~acid~~ **base**, relative to ~~RNA~~ **DNA**, is due primarily to the presence of a ~~3'~~ **2'** hydroxyl group on ~~DNA~~ **RNA**, which is not present in ~~RNA~~ **DNA**.

e) **TRUE** DNA utilizes the nitrogenous base "T" because any "U" created from deamination can quickly be identified and removed.

2. We learned in class about a method, developed by David Tirrel and co-workers at CalTech, for incorporating unnatural amino acids that makes use of endogenous cellular machinery to load unnatural amino acids in place of natural amino acids. One example of this is the incorporation of the unnatural amino acid azidohomoalanine (shown below) in place of the natural amino acid, methionine.



methionine azidohomoalanine

a) Based on your understanding of the process by which natural amino acids are loaded, or charged, on to tRNAs, describe the enzymatic/chemical steps required for charging a tRNA with azidohomoalanine. (4 points)

1- activate the amino acid, using ATP

2- transfer the active amino acid to tRNA's 3' OH group

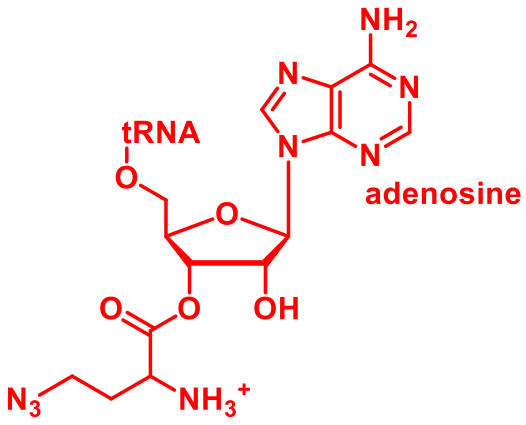
b) What is the name of the enzyme that catalyzes the process described above? What is the sequence of the anti-codon that codes for azidohomoalanine (write out in 5' to 3' direction) (4 points)

amino acyl tRNA synthetase (or tRNA synthetase)

codon = 5' AUG 3', so the anticodon would be 3' UAC 5'

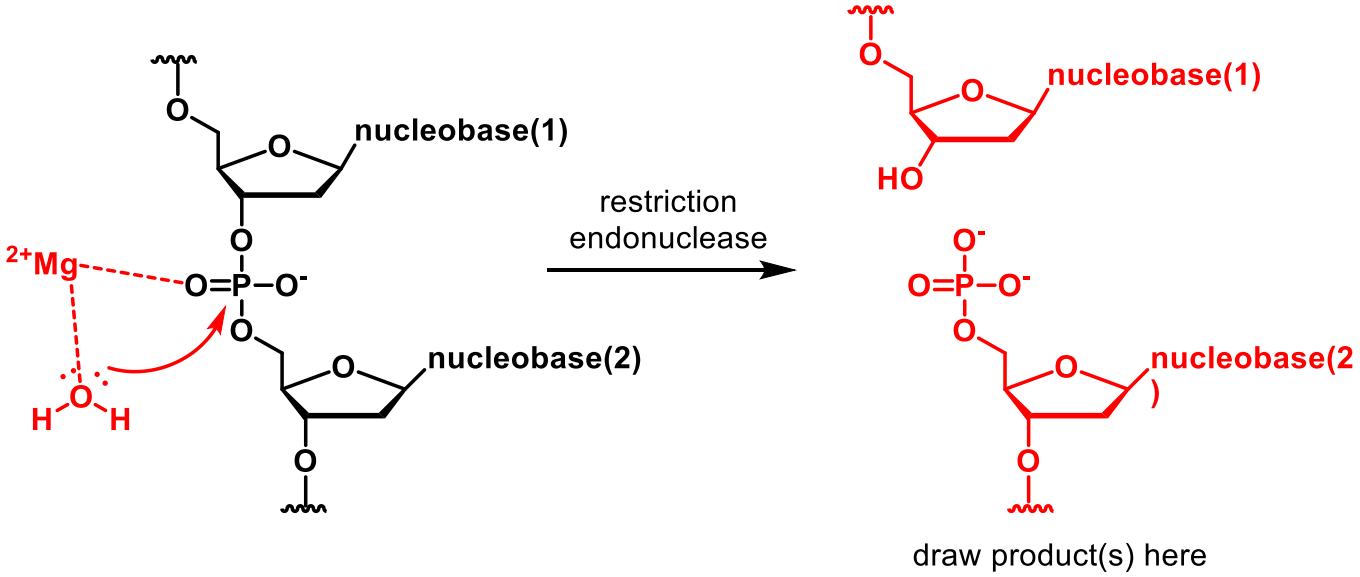
answer: 5' CAU 3'

c) Provide a detailed drawing of the linkage between tRNA and azidohomoalanine. Be sure to indicate the structure and identity of terminal nucleotide that links to the aminoacid. (12 points)



3. Restriction endonucleases are a class of enzymes that recognize and cleave the phosphodiester bond of dsDNA.

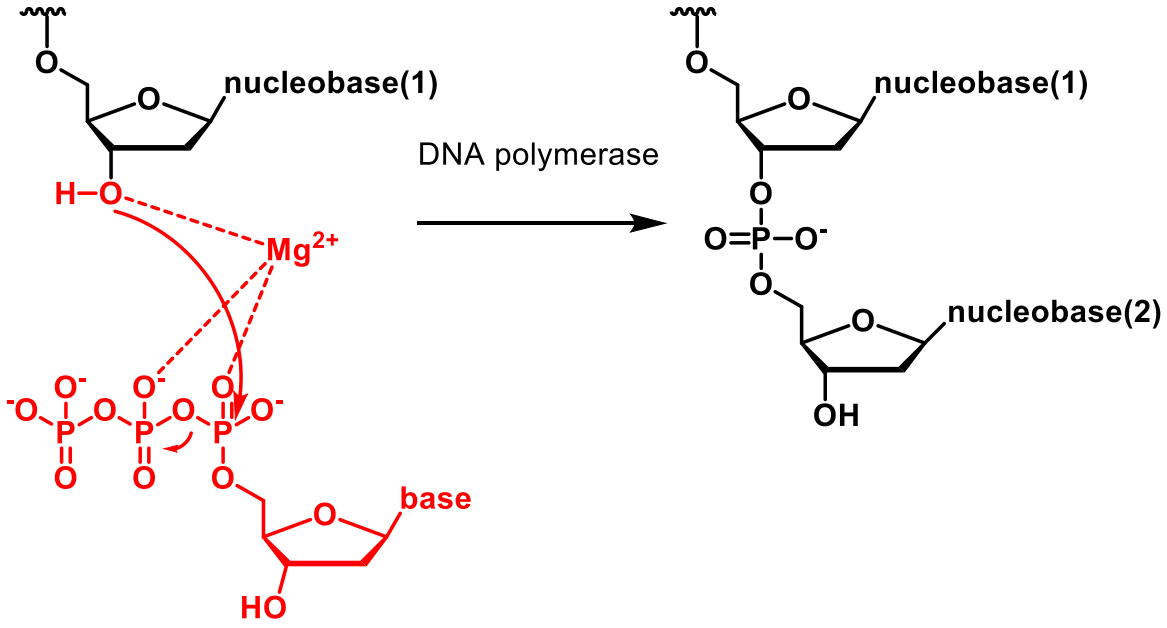
a) In the space below, please indicate what the products of the reaction catalyzed by a restriction endonuclease would be. (6 points)



b) Additionally, on the left hand side of the reaction arrow, indicate what the nucleophile would be that would give the products you drew. Draw in the appropriate arrows on the structures above to show where this nucleophile would attack. (6 points)

c) Based on your knowledge of course material, what additional co-factor/reagent is used by restriction endonucleases to facilitate the cleavage of the phosphodiester bond? Draw in this co-factor and show how it might interact with the substrate and nucleophile. (6 points)

d) In a complementary reaction, DNA polymerases help to catalyze the formation of phosphodiester bonds. Shown below is a partial description of this reaction. Please draw in the missing reagents/substrates that are required to form a new phosphodiester bond. Please make sure to draw in the appropriate functional groups on the ribose on the reactants side – this has been left incomplete. (6 points)

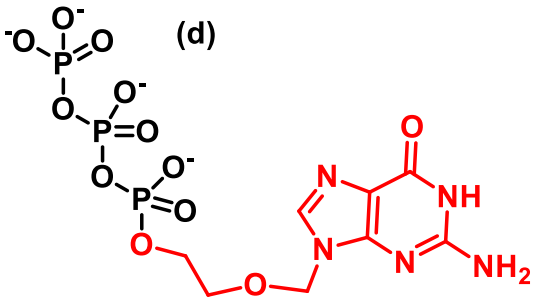


draw reactants here

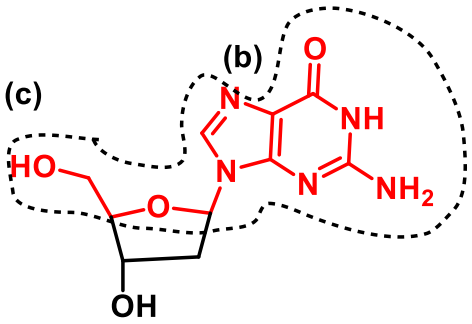
e) As you did for the restriction endonuclease example above, draw in arrows on the structures/space above to indicate the identity of the appropriate nucleophile and electrophile for this reaction. (4 points)

f) DNA polymerase uses the same co-factor that you identified in part c) of the restriction endonuclease example. Be sure to add this co-factor to your drawing in part d) to show how it helps to facilitate the reaction catalyzed by DNA polymerase. (6 points)

4. The molecule below, acyclovir, is a potent anti-viral compound with efficacy against herpes virus.



acyclovir



(a) guanosine

Name/Structure

a) In the space above, provide the name of the nucleoside that most closely resembles the structure of acyclovir. (2 points)
b) In the space above, provide the structure of the nucleoside (ribose + nucleobase) you indicated in part (a), above. (2 points)
c) On the structure of the ribose you drew above, indicate which portion is most similar to acyclovir. (4 points)
d) Acyclovir is a great substrate for viral kinases, which phosphorylates acyclovir to generate acyclovir monophosphate. The monophosphate of acyclovir can be further phosphorylated to generate acyclovir triphosphate. On the structure of acyclovir above, draw in the modification to make acyclovir triphosphate. (3 points)

e) Acyclovir triphosphate is a competitive inhibitor of a herpes virus enzyme. In the presence of acyclovir triphosphate, viral genome replication is substantially impaired. Provide the name/class of the enzyme that is the likely target of acyclovir triphosphate. (3 points)

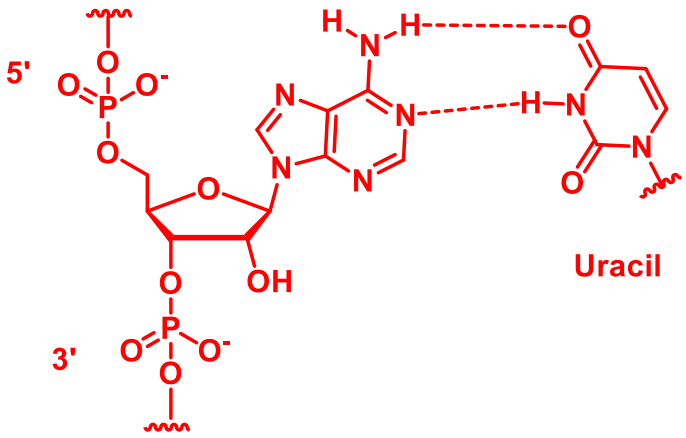
DNA polymerase (III) (targets *replication*)

f) In addition to acting as a competitive inhibitor of the enzyme you identified in part (**e**), how else might acyclovir triphosphate be a potent disruptor of viral genome replication? (1 to 2 sentences) (4 points)
Acyclovir triphosphate would act like a replication terminator. It could like add to the growing 3' end of a replicating DNA strand, but then would not be able to allow additional NTPs to be added, since it has no 3' OH itself.

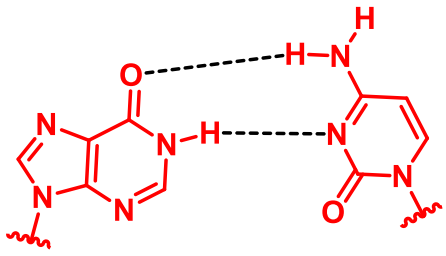
g) We discussed a number of applications of the enzymes related to the Central Dogma: PCR, Sanger Sequencing, Directed Evolution, Unnatural Amino Acid incorporation. The mode of action of acyclovir that you described in part (f) is mostly similar to which of these topics? Explain your choice in 1-2 sentences. (2 points)
Sanger Sequencing. Acyclovir triphosphate acts like a ddNTP to stop DNA synthesis.

5. A special class of enzymes that edit RNA are called ADARs: adenosine deaminases that act on RNA. ADARs convert "A" residues in RNA into inosine. Defects in ADAR function have been linked to a variety of human diseases, including cancer, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease.

a) In the space below, draw the H-bonding interactions between the A residue in mRNA and the corresponding Watson-Crick-Franklin based pair in the anti-codon of a tRNA. Please provide a clear drawing of the nucleobases. You only need to draw the ribose and phosphodiesters for one of the bases. (10 points)



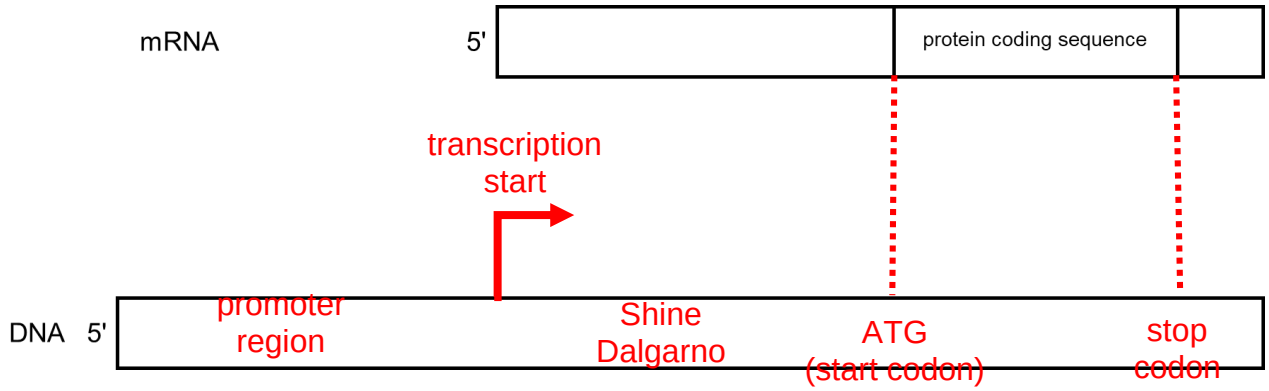
b) When ADAR catalyzes the deamination of A on the mRNA, it generates a new nucleobase in its place, inosine. Does inosine have the same preference for Watson-Crick-Franklin basepairing as "A"? Answer this question and draw in the nucleobase that participates in the best Watson-Crick-Franklin H-bonding with inosine. Please show the key H-bonding interactions between inosine and this nucleobase. Since you drew in the ribose and phosphodiesters in the answer above, you may just draw in the nucleobases here (but please indicate the location of the glycosyl bond). (9 points)



c) The change from "A" to inosine could result in a change at the amino acid level of the protein encoded by this mRNA transcript. Would the "A" to inosine change be likely to have the greatest effect on the protein encoded by the mRNA if it was at the 1st or 3rd position of a particular codon? (1-2 sentences) (4 points)

This would have the greatest effect in the first position, because the 3rd position of the codon is the wobble position and doesn't contribute as much to the specificity of the anti-codon binding. Therefore, mutations at the 3rd position would be less like to result in an amino acid change.

d) Suppose the mRA transcript containing the "I" was shown below. Below this mRNA transcript, draw out a scheme of the coding strand of DNA. On the coding DNA strand, indicate the location and identity of the start codon, the approximate location of the Shine-Dalgarno sequence, the transcription start, the promoter region, and the location (not the identity) of the stop codon. (8 points)



6. Over the summer you and your classmates isolate a new strain of bacteria from the fountain in the chemistry plaza (just outside Latimer 120!). Separately, some friends who had explored Yellowstone National park over the summer return back from their trip with a new strain of bacteria they isolated from a hot spring (water temperature >70 °C!). In your excitement to compare the two different strains, the labels on the tubes were mixed up! Fortunately, you were able to determine the relative base pair composition of the DNA of each of the two new, unidentified strains of bacteria. Assuming these bacteria possess dsDNA genomic DNA, answer the following questions.

a) **Strain A** has 36% "A" content. What is the relative composition of the other base pairs? (3 points)

36% A → **36% T** = 72% A-T

100-72 = 28% GC => **14% G, 14% C**

b) **Strain B** has 18% "A" content. What is the relative composition of the other base pairs for **Strain B**? (3 points)

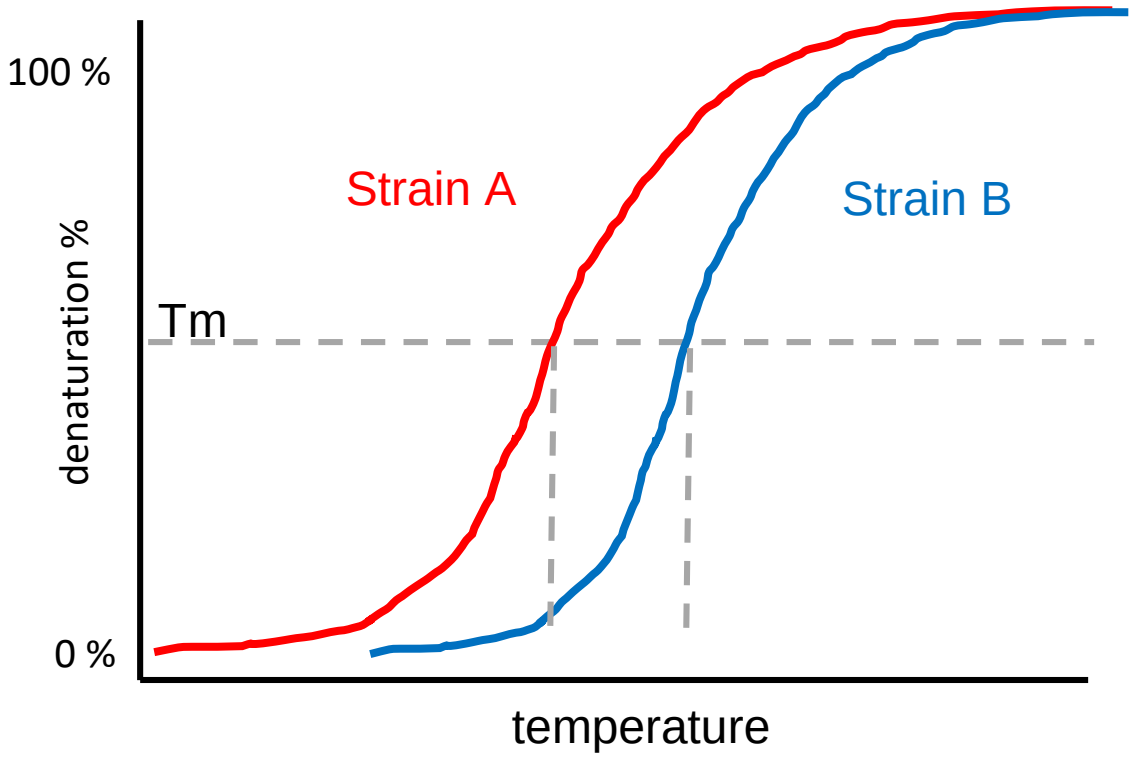
18% A → 18% **T** = 36% A-T

100-36 = 64% GC => **32% G, 32% C**

c) Briefly explain your reasoning for the answers you provided in parts a and b. (1-2 sentences) (3 points)

The relative amounts of A = the relative amounts of T. The total AT content + total GC content will add up to 100%

d) In the space below, plot a melting curve for the genomic DNA isolated from **Strain A** and **Strain B**. Please label the x-axis and indicate on the plot how you would determine the melting temperature of the DNA for Strain A and Strain B. You do not need to provide actual melting temperature values, only indicate the relative positions of the melting curves. (5 points)

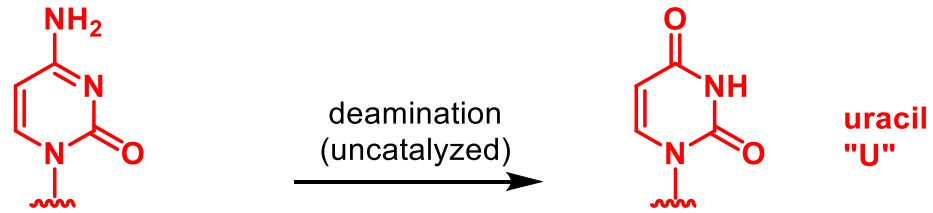


e) Based on your answers from parts **a-d**, which strain do you think was isolated from the hot spring at Yellowstone. Briefly explain your reasoning. (1-3 sentences) (3 points)

Strain B is from the hot spring. A higher G-C content gives it a higher Tm, which would be needed in the warm environment to make sure that the dsDNA of the genome didn't melt.

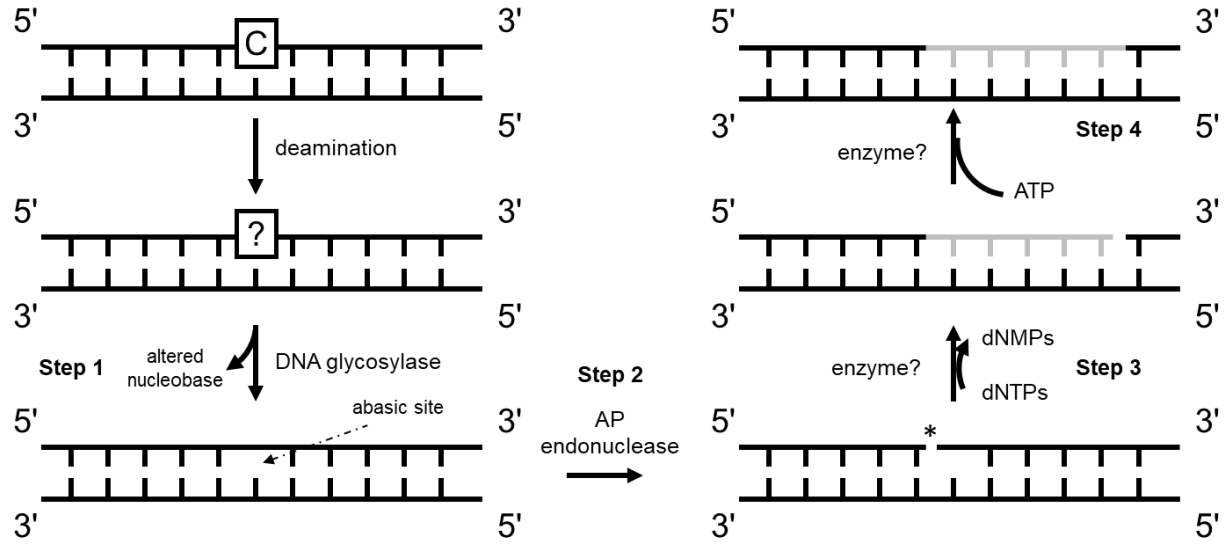
7. Cytosine slowly undergoes a process known as deamination. This changes the identity of cytosine.

a) Below, please draw out and name the structure of both cytosine and its deamination product. You only need to draw the nucleobase, not the ribose (please indicate where the glycosyl bond would be, however). (6 points)

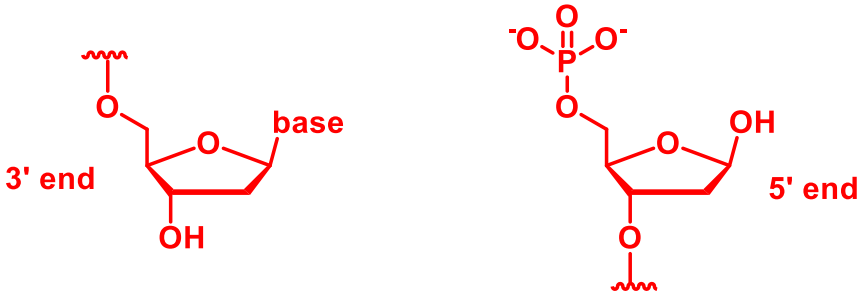


cytosine product

The resulting change in cytosine results in a basepair mismatch. In cells, a process called base excision repair takes over to fix this mistake. Shown below is a schematic of this base excision repair pathway.



b) In the first step (**Step 1**) a DNA glycosylase specifically recognizes and removes the altered nucleobase you identified as the product of cytosine deamination, leaving behind an abasic site (i.e. just the ribose). In the second step (**Step 2**), a special endonuclease, AP endonuclease, which recognizes abasic sites, cleaves the phosphodiester bond on the 5' side of the abasic site, leaving a nick in the phosphodiester backbone (indicated by a *). If AP endonuclease uses the same catalytic mechanism as restriction endonucleases we discussed in class, please draw out what the structures of the newly created 3' and 5' ends look like. You only need to include the ribose and phosphates, no nucleobase. (6 points)



c) What co-factors/metal ions, if any, do you think are likely to be utilized by AP endonuclease? (2 points)

Magnesium

d) In the third step (**Step 3**) of base excision repair, a new enzyme adds dNTPs to the 3' end of the indicated strand (the newly added bases are light grey in the figure). What is the name of this enzyme? (2 points)

DNA polymerase

e) The process of base excision repair is finished when, in **Step 4**, a final enzyme repairs the phosphodiester backbone, using one equivalent of ATP, to create one contiguous DNA strand. What is the name of this enzyme? (2 points)

DNA ligase

f) **Steps 3** and **4** of base excision repair are most closely related to what process we discussed in lecture? (4 points)

cleanup of Okazki fragments / cleanup of lagging strand of replication

g) Deamination of cytosine (part a) requires DNA repair via the base excision pathway to repair this mistake. If the modified base is not repaired to "C," what is the consequence for the organism? (1-2 sentences) (4 points)

If U remains, it pairs with A, instead of C to G. This would result in a G to A mutation in the DNA upon replication.