

Will the exam be in the same room and at the same time as the lectures?

Yes.

So are we going to be tested on the 20 amino acids for this exam?

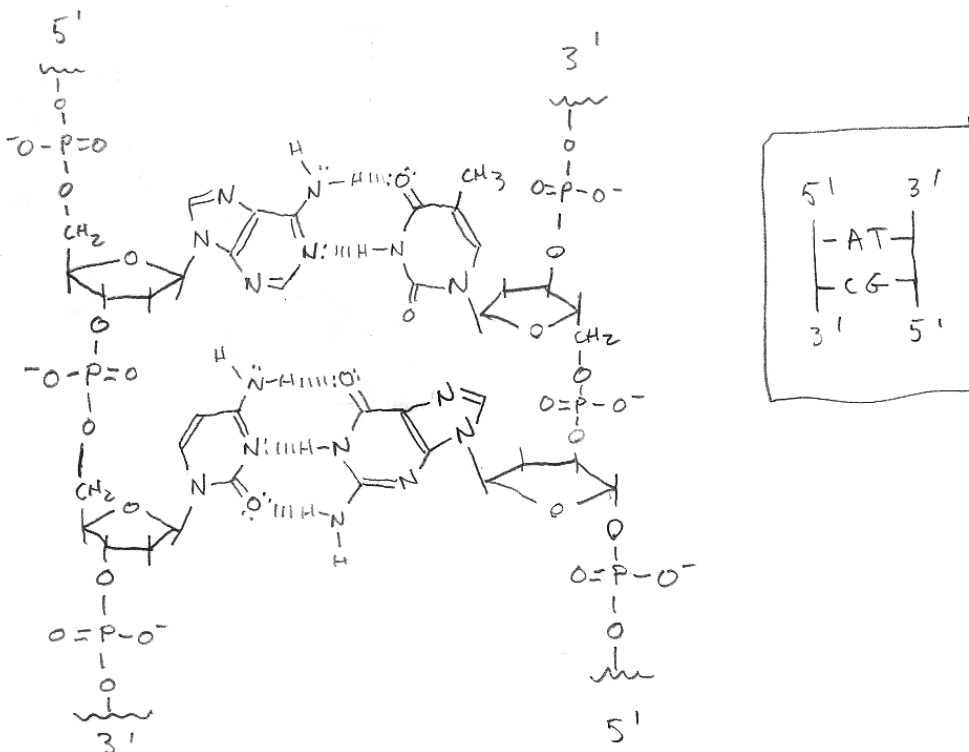
Sorry, but no - not until we cover protein biosynthesis. However, you should know the few we have seen in the lectures.

Is a phosphoester bond considered a type of glycosidic bond?

Yes, because any bond to a sugar is, but generally when you refer to THE glycosidic bond in a nucleotide you are referring to the sugar – base bond.

Hi professor, I was trying to draw a DNA strand with its complementary strand with H-bonding but the H-bonding wasn't pairing up. What I was trying to do is draw 5-3 strand and then it's complementary 3-5 strand upside down but the H-bonds to the right T-A and C-G bases wasn't matching up correctly. Could you maybe please post a sample of maybe 2 or 3 bases and their complements with the sugar-phosphate back bone. Thanks.

Good idea, see below: (Remember the real thing is in three-dimensions, so you have to draw it a little weird)



I'm still a little stuck on uracil in DNA. You said that if uracil is present in DNA it's mutagenic and the cell has no way of repairing or even recognizing that a mutant is present. If the cell doesn't recognize uracil as mutagenic and has no repair mechanism against it, then why is it mutagenic? Also you used cytosine as an example, deamination of cytosine produces uracil. Why was that example given, is this the only way that uracil can be made? Can demethylation of thymine also produce uracil? Please help Pete.

Okay, THIS IS IMPORTANT:

I didn't say that if uracil is in normal DNA that there is no way to detect it. In fact if uracil is present in DNA, this is a big mistake that the cell does recognize and can easily repair. What I described in class was a purely HYPOTHETICAL situation where uracil was used by the cell instead of thymine in DNA and why this would lead to more mutations.

Hypothetically, if uracil was in DNA (let's say we only have uracil and no thymine anywhere) when the deamination of cytosine would happen, we would generate uracil in its place. If we already have uracil in the DNA, than there is no way of telling if the new uracil is a mistake or not (of course looking at the base it is paired up with could be an option, but who's to say the other base isn't the mutated base).

In reality, when the deamination of cytosine occurs, this produces uracil and the cell recognizes this as a mistake. This C → U mutation is fairly common. As you say in your question, thymine would become uracil if you demethylate it, however, this mutation is vanishingly rare, especially when compared to the C → U deamination.

I've learned about DNA a lot, and I never heard about the propeller twist till yesterday, which was pretty cool. Does it (especially the 1 degree one in BDNA) happen because of attractive/repulsion forces between the base pairs that neighbor above and below? Or does this naturally happen due to the twisting helical structure of DNA? After looking at the A-DNA and how the angle increases with more twisting, my guess would be that the natural twisting would be the cause of propeller twist.

You are exactly right with the second statement. The larger helical structure can cause imperfections throughout the molecule. An important thing to remember is that the double helix is fairly flexible - twisting, bending, etc - and any changes to the global shape will have an effect on the local structure of the bases, sugar-phosphate backbones and the minor and major grooves.

Do we need to memorize the names of the reactants, products, cofactors, and enzymes?

For sure. For the pathways, I generally care more about the structure and the reaction than the names of the molecules or enzymes (but you should certainly be able to name the major molecules and enzymes).

Which THF derivatives should we know?

Know the ones from the lecture. We saw THF, N¹⁰-formyl-THF, N⁵,N¹⁰-methylene- THF and DHF (dihydrofolate), the oxidized form of THF.

When IMP tacks on the Asp to become Adenylosuccinate, is there a water molecule produced as the formerly carbonyl oxygen leaves? It seems like there would be, but it's not mentioned on the notes, and I have not taken chemistry in a while. So I could be missing something. Thanks!

Good question. It would, but notice there is also a molecule of GTP that is hydrolyzed to GDP + Pi during this reaction. The hydrolysis requires a water molecule, so there is no net production of water.

On page 8 of lecture 2 notes, bottom left, that should be an NH₂, right?

Yes, of course. I accidentally left out the hydrogens on that amide.

On page 14 of the Nucleotide Metabolism lecture you talk about forming deoxyribose nucleotides; we need them in their Diphosphate forms, but then when you convert dUMP to dTMP, it is in the monophosphate form. So it has to be in the monophosphate form? Could you elaborate on this a bit please?

Good question. First of all, the ribonucleotide diphosphates are used to make the deoxyribonucleotide diphosphates. So you can see how we get dUDP from UDP. From there, we need to be in the deoxyuridine monophosphate (dUMP) form if we are going to make deoxythymidine monohosphate (dTMP - see the reaction catalyzed by thymidine monophosphate synthetase in the notes). To get dUMP, the enzyme dUTP diphosphohydrolase (or dUTPase) will produce dUMP and PPi. This is a weird reaction that I didn't spend much time on in class (weird because it cleaves a pyrophosphate instead of transferring these phosphates to another nucleotide). The following would be a good series of reactions:

(I put the molecule we are following in bold)

UMP + ATP → ADP + **UDP** (enzyme: specific monophosphate kinase)

UDP + NADPH + H → **dUDP** + NADP⁺ + H₂O (enzyme: ribonucleotide reductase)

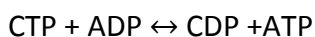
dUDP + ATP → ADP + **dUTP** (enzyme: nonspecific diphosphate kinase)

dUTP + H₂O → **dUMP** + PPi (enzyme: dUTPase)

dUMP + N⁵,N¹⁰-methylene THF → **dTMP** + Dihydrofolate (enzyme: deoxythymidylate synthase)

In lecture #2 slide 12, you show us the synthesis of CTP from UMP. But on slide 13, where you synthesize deoxyribose nucleotides, it says it requires the diphosphate form. How did you convert CTP to CDP (with nucleoside diphosphate kinase?)? It wasn't specifically shown at this part of the lecture.

Yes, using the diphosphate kinase is one way. Here is the reaction:



Can you explain why Pol I can't fix the nick b/w DNA b/c there's only one phosphate instead of triphosphate. I guess i'm not understanding why it needs triphosphate to elongate. It normally just attacks the first phosphate right? What happens to the other two?

The phosphodiester that the DNA polymerase normally creates with the incoming nucleotide and the 3' end of the growing polymer requires there be an energy source in the form of the three phosphates (hydrolyzing off the pyrophosphate and then hydrolyzing it). If there is a nick, then there is only a single phosphate and no way for a phosphodiester linkage to be formed by a polymerase. This is why ligase is necessary.

Just confirming that Dna Polymerase I is the only polymerase involved in exonuclease activity correct? Polymerase II and III have nothing to do with nuclease activity correct?

This is not correct! Pol III also has 3' → 5' exonuclease activity for error correction (proofreading). Pol I has both 3' → 5' and 5' → 3' exonuclease activity. The 5' → 3' activity is how it removes the RNA primers.

Also regarding the histone proteins, do we need to know any details about H1, H2A, H2B, H3, H4 other than that they are histone proteins?

Remember what we said about their physical properties: that they are basic, what amino acid residues they contain, and how we can modify their DNA binding properties.

What is important about the open complex in replication?

This is what recruits Pol III so that polymerization can begin. Remember, the Polymerase doesn't just bind to BDNA and start rolling. It needs to have access to single stranded template DNA.

When hydrolyzing the phosphodiester bond during 3' → 5' exonuclease activity, I noticed that only one phosphate group leaves with the wrong base (for example dCMP), what happened to the other two phosphate groups (bc require dCTP at the beginning)? And how does it leave when the right dNTP comes in?

There is only one phosphate because even though it is the wrong base, a phosphodiester link has been made and pyrophosphate has been lost. This bond must then be hydrolyzed by the 3' → 5' exonuclease activity of the polymerase, and the wrong base is then released in the monophosphate form. Once it is gone a new residue can get into the active site.

DNA is usually negatively supercoiled. Is this so that it is easier to separate the helix?

Exactly.

I wanted to see if you could explain the mechanism of ligase please! I know what it does, but I'm confused about how it actually works, and the book didn't help.

Ligase first becomes activated by adenylating itself. It then attaches the AMP to the free phosphate end of a nicked strand forming a 5' to 5' link. The purpose of this is to generate a phosphoanhydride bond, which is used to drive the removal of AMP. As AMP is removed the formation of a phosphodiester linkage occurs, fixing the nick.

Is Right-handed supercoiling of circular DNA the same as NEGATIVE supercoil and vice versa? I'm confused about this part bc i know that to get a R-hand supercoil, you introduce a negative turn (clockwise) and to get a L-handed supercoil, you introduce a positive turn (counterclockwise). But im not sure what it means to be one or the other. Which form allows the DNA to separate easier?

Yes. The negative turning (unwinding, negative supercoils) is what allows the DNA to separate easier and causes the supercoils to have a right handed twist.