

Are the slides titled "DNA replication," and "DNA replication 2" new slides posted after the first midterm? If so, are any of these slides going to be tested on the second midterm?

These will not be on the exam.

Also, what about the topoisomerase slides?

Yes. We (royal) agreed it would be included for this exam. It is easy material to learn, so it should make the exam easier overall!

My question is regarding about the direct repair (pyrimidine dimers). Should we memorize the whole process of pyrimidine dimers repair with photolyse including the pictures that is on our lecture note?

No. Just know what the cofactors are, and overall what is going on.

I was going through the transcription notes and had a question. Last class you made a statement regarding Page 6 of the transcription notes. Something regarding the use of + vs - supercoiling and the winding/unwinding diagram. I have so many things written on that slide now that I am kind of confused on what I wrote down. Can you tell me what the take home statement was regarding the coiling and the enzymes used for the diagram? I would really appreciate it.

The DNA is unwound at the polymerase. As the polymerase moves (the unwound part moves with it), it causes positive supercoiling ahead of it, but negative supercoiling behind it.

Polyadenylate polymerase does not require a template but does require a "primer." Is this a different sort of primer than what we normally think of as a primer? Because I usually think of a primer as a stretch of RNA that provides a 3' end to add to, but when we are adding the poly-A tail it seems like we already have our 3' end. So here, is the "primer" a "premade" stretch of -AAAAAAAAA to stick on the end to start off the addition of more ATPs? (I did look in Voet and online and wasn't able to figure this out.)

Primer here simply means a 3' OH to extend. Thus *the transcript is the primer* because it has a 3' end. There are no extra nucleotides added on to serve as a primer.

I don't see the difference between where the two cleavage sites are during mismatch repair (exonuclease VIII/RecI nuclease vs. exonuclease I/exonuclease X).

The difference is which side of the mismatch the removal takes place. One is a nick to the 5' side of the mismatch the other is a nick to the 3' of the mismatch. Don't worry about which system goes with which, just know the big picture of what is happening and the generic functions of the enzymes (e.g. exonuclease, helicase).

U1 binds both the 5' and the 3' splice sites, but I can't figure out when exactly the 3' binding occurs. Is it after the U1 is kicked off from the 5'? I found one summary of a pubmed article that mentioned that some of the RNA on the U1 matched with the 3' end and assisted in splicing, but there was no discussion in the summary beyond this (particularly when the binding occurs) and I couldn't get the full article to see if it was discussed in there.

Remember, the splicing snRNPs *assemble first into an inactive complex* to prevent premature cutting of the transcript. Once everything is in the right place, *there is a rearrangement to the active complex* which does the splicing. Don't worry about the details of the 3' binding by U1 for this class.

The Mut H is what cleaves the unmethylated strand during mismatch repair (cleaves once it figures out which is the unmethylated strand)...correct?

Yes.

You mentioned Pol I can't go backwards but I thought it had 5'→3' and 3'→5' activity...

It has 3'→5' exonuclease activity, but it only goes backwards for a single nucleotide. It doesn't remove long stretches of bases in the 3'→5' direction.

Why is it that it is easier to control prokaryotic transcription vs translation?

This is a good question. Prokaryotic mRNA is not very long lived, so it doesn't hang around very long. To keep enough mRNA around to have it ready for when the protein is needed would be very wasteful for the cell.

What is galactose used for? Do we need to know?

Galactose, like glucose, can be metabolized as a carbon source and energy source. The main pathway for its metabolism is glycolysis.

With "gene on", if the cell begins producing its own TRP because no external source is present, will the produced TRP bind with the repressor protein and the complex bind to the operator and turn the gene "off"?

Yep. That is feedback inhibition.

An operator is the same thing as a control site right? and it's located in the promoter?

Yes, but it can be located outside of the promoter as well.

Are the genes linked in polycistronic mRNA associated with similar proteins?

Usually the genes are related to a common function, such as the biosynthesis of tryptophan.

Is the IPTG used in labs to activate the lacOperon a derivative or related to the thiogalactoside transacetylase?

The IPTG has nothing to do with the enzyme thiogalactoside transacetylase. It is only a laboratory equivalent of allolactose that is added at the point when you want to 'turn on' your gene which is under control of the lac promoter.

On the first lecture, we discussed footprinting- can you go over the process and why does it say "add polymerase but do not add RNTPs (polymerase can't move)? Why do we use footprinting?

If you let the polymerase bind to the promoter without adding RNTP's, the polymerase can't polymerize RNA, so it just sits there. If you then add enzymes that chew away DNA, everything will be removed except the area that is covered by the polymerase. This is how you can locate the promoter (one reason to use footprinting).

Are post-transcriptional modifications only for eukaryotes?

If we're talking about mRNA's only, then yes. But, even prokaryotes modify their tRNA's and rRNA's.

DNA is only in the "hemimethylated" stage for a short period of time right? because eventually both strands will be methylated? So repair enzymes only have a short period to spot the mistake and correct it right?

That is correct. It takes time for methylation to 'build-up' on a strand. For this reason repair enzymes have a window of time to work.

Lariat Formation: Just to clarify... The odd bond is the 2'→5' phosphodiester bridge, but is correct that the lariat loop is a 3'→3' Bond?

The 'Y' in the lariat loop has the normal 3'→5' linkage, but it also makes a 2'→5' linkage with its free 2' OH group. No 3'→3' linkage!

In nucleotide excision repair, why are ~12-13 bases removed from the damages strand (including the TT dimer) instead of removing the TT dimer alone?

The size of the piece that is removed is very small. The fact that a few bases on either side of the dimer are removed does not really matter.

Do we have to know the function of the individual subunits that make up the prokaryotic RNA Polymerase?

Just the ones we discussed.