1. DNA repair (36 pts):

(a) 15 pts) Draw the structure of an abasic site in DNA. This is the common intermediate for what type of DNA repair? Given that this type of repair exists, and that it's much simpler than NER, why do you think NER evolved as well?

Score for the page__________
(b; 6 pts) What is the source of the information needed for error-free DNA repair via (1) direct repair, (2) NER, and (3) DSB repair? (Very short phrase each)

1. It's built in to the (w)ren.
2. The other strand of the DNA.
3. Another copy of the old DNA, like a sister chromatid.

(c; 15 pts) Sketch the MutS-HL complex on DNA just after incision, and indicate the function of each protein. We argued that through-solution looping does not make sense as a mechanism for establishing the MutS-HL complex, that tracking makes more sense. What is the reasoning? (Note that while there's some experimental evidence for tracking, this is not a done deal.)

- System needs to know which direction to travel to remove the daughterstrand DNA. A through-sense loop could form with MutH in either orientation.

Speed: +1/5

Score for the page: ___________
2. Translation (26 pts):

(a; 9 pts) In kinetic proofreading during aa-tRNA selection on the ribosome, there is a branched pathway for acceptance vs. rejection. What is the acceptance branch called and what happens during acceptance? What is the rejection branch?

\[ \text{Acceptance} = \text{Accommodation} \]

\[ \text{Rejection} = \text{Dissociation of aa-tRNA after ATP} \]

\[ \text{Hydrolysis} \]

The existence of tradeoffs among speed, energy cost, and fidelity is a general theme of biological information processing. Translation offers an example. Streptomycin is an antibiotic that inhibits growth by markedly increasing the error rate of tRNA selection in translation. The mechanism is complex but the bottom line is that *kinetic proofreading becomes much less effective at rejecting mistranslated tRNAs.*

(b; 6 pts) Why would an increased error rate for protein biosynthesis hurt the cell (not a trick question)?

What name did we give the corresponding effect of inaccurate DNA polymerases?

\[ \text{A lot of bad protein would be made} \]

\[ \text{The error catastrophe / Hyper mutants} \]

\[ \text{Homologous recombination} \]

(c; 8 pts) Streptomycin-resistant (SmR) ribosomes are hyperaccurate, i.e. they make fewer mistakes than wild type ribosomes. Recall that hyperaccurate DNA polymerases (anti-mutators) have very active \[ \text{S} \rightarrow \text{S}^* \text{ Exonucleases} \] (fill in the blank). Analogously, how might the EF-Tu-GTP-tRNA kinetic proofreading mechanism above be altered to make a hyperaccurate ribosome?

\[ \text{Rejection of tRNA could be accommodated faster, to} \]

\[ \text{Reduce the chance that a mischarged tRNA would have time to accommodate. Increased rate of rejection maximizes fidelity.} \]

\[ \text{(Other possible answer)} \]

\[ \text{Faster GTPase} \rightarrow \text{fewer events} \rightarrow \text{less time allowed for accommodation} \]

Score for the page:__________
(d; 9 pts) The SmR ribosome is not the wild type: in other words, hyperaccuracy is actually not selected for in the absence of the antibiotic. Give two possible reasons, one based on economy and one based on speed; we will not consider the possibility that the errors per se can be useful. Based on general themes from the course, is the argument from economy or the argument from speed more likely to be correct?

- Economy: increased fidelity wastes GTP
- Speed: overall rate of translation will be decreased because rate of rejection of wrong tRNA

(+3) Speed - tRNA are not limiting for growth

(+1) Fidelity is expensive.

(c; 5 pts) Some SmR mutants are actually streptomycin-dependent (SmD), i.e. they die in the absence of the antibiotic. Why might this happen?

(+3) Balance of the two effects. The drug speeds up translation. The SmR ribosome stabilizes tRNA on ribosome. The ribosome lab is so slow enzyme that tRNA is not translated rapidly enough to live. In the absence of drug, translation is so slow bug can't live.

Score for the page__________.
3. RNA Biology (22 pts):
(a; 15 pts) Fill in the boxes in the schematic at the right of three possible outcomes of RNAi in plants.

(b; 6 pts) How might RNAi have evolved? Hint: administering long dsRNA molecules to mammalian cells sets off the interferon response. Could have been an anti-viral response to destroy dsDNA virus invaders.

(c; 9 pts) What are two of the main attractions for oligonucleotide-based therapeutics as opposed to e.g. small-molecule inhibitors of enzymes? What is one serious difficulty with administering oligonucleotides to patients?

+3 Can knock out protein completely instead of just its activity - longer lasting effect?

+3 Any target can be approached with a smaller molecule

+3 Using the body's own machinery to amplify signal.

+3 They are big, charged molecules - hard to get into the cell, subject to nucleic acid activity.

Score for the page__________
(d; 10 pts) RNA has been suggested as the primordial self-perpetuating macromolecule, because it can combine information carrying and catalytic function. In the last few billion years, RNA has specialized mainly in transesterifications of one kind or another. Why has DNA taken over information storage? Why has protein taken over most catalytic functions? Why does RNA retain primacy in nucleic acid transactions like splicing?

13 - DNA is more stable, can have larger genome
13 - Protein have much more versatile functional groups
14 - RNA is good at base pairing to recognize substrate and/or given reaction, and/or good at many substrate around

3. Eukaryotic Transcription (27 pts):
We've seen that eukaryotic transcription involves combinatorial regulation by sets of transcription factors, and that chromatin remodeling activities are recruited as part of the process.

(a; 6 pts) Wouldn't it be simpler to have a single transcription factor dedicated to each gene? Give two simple reasons hasn't life evolved this way?

13 - Would need one factor per gene - and who would regulate the regulators?
13 - This would not allow for integration of signals.

Score for the page:________
(b; 12 pts) In general, give two ways in which one transcription factor can potentiate the activity of another, and two ways in which one can repress the other. Some possible activate/repress mechanisms rely on similar mechanisms, that’s okay.

Activates:
1. Cooperative binding
2. Recruitment of chromatin remodeling activity that allows binding of the other one
3. Formation of a common recruitment intermediate

Repress:
1. Block binding of the other factor
2. Recruitment chromatin to block access
3. Disruption of common intermediate
4. Alter DNA structure to separate factors

(c; 9 pts) How can SWI/SNF ATPases either activate or repress transcription depending on the gene in question? How might they end up repressing some genes even if locally they could only act to activate transcription?

What do SWI/SNF ATPases do?

- Flexibly nucleosomes - move them around
- They can slide over the binding site for a transcription factor
- They could activate a TF repressor or target gene

Score for the page__________
4. Connections and Miscellaneous (29 pts)
(a; 24 pts) We have seen several examples of potentially dangerous macromolecules with cryptic activities that are delivered or activated by molecular matchmaking. Describe two examples: for each, list (1) the molecule with the cryptic activity, (2) the nature of the activity (i.e. a restriction enzyme, which is not otherwise an answer I am looking for, would be described as "cuts DNA at a palindromic recognition site"), (3) the partner that loads/activates the molecule from (1), and (4) one reason that molecule from (1) doesn’t just do everything itself without help.

1. Mut H
   U6 in Rep
   Uvr BC

2. nuclease
   Rmt with
   cleaves/larging
   nucleen

3. Mut L
   U4/U5/U6 from Rep
   Uvr (A,C) B

4. Wouldn’t want nuclease activity running around the cell, or motocompete spaying

(b; 5 pts) Suggest an improvement I could make in the coverage of a topic of your choice.

men up to date with mutshi

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Score for the page__________

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