1. Suppose Griffith had make his observations using heat killed S1 and R1 strains of the Pneumococcus. Would he have seen the same results as far as pneumonia in mice? What control experiment would be required for the results to be interpreted?

DNA from the heat killed S1 could enter the R1 cells and transform them to S1, thus some mice would develop pneumonia. He would have to demonstrate that the frequency of transformation is greater than the rate of reversion (back mutation) from R1 to S1.

2. In the 1980s, E. coli strains with a temperature sensitive mutation in the gene encoding topoisomerase I were reported that quickly stopped DNA replication when switched from 27 to 37°C. Similar experiments identified temperature sensitive mutations in the gene encoding topoisomerase IV. However these mutants completed replication of the chromosome but could not divide.

   a) Speculate as to the role for the Top1 and Top4 genes.
   Top1 must be directly involved in resolving supercoiling ahead of the DNA replication fork.
   Top4 is most likely involved in the separation of the two DNA daughter helixes following replication.

   b) When the Top1 mutants were kept at 37° for some time, some cultures began to grow again, but the Top1 defect was not reverted to normal. What other genes may have changed to suppress the effect of the Top1 defect? (include at least one that is not a topoisomerase gene.)
   Other Top genes have assumed/filled the role of Top1. A likely non-Top gene would be gyrase. Suggestions of suppressor tRNAs etc. were accepted.

3. In studies with bacteria in those cases where only two codons ending with U or C code for an amino acid (such as those for Phe, Asp and His) in the great majority of the time, the codon ending in C was present in mRNAs. Do you think this is just ‘chance’ or might there be a more logical explanation? Explain your choice.

   Most likely not due to chance. The most probable explanation in my mind (and in the literature) is that Wobble has something to do with it, in that a G in the anticodon wobble position will pair with C or U, but the affinity is better with C and there are more of these tRNAs for the lys tRNA that would be. The anticodon ending in A would only recognize the U ending-codon but it may be rare. Explanations based on DNA being more stable with GC base pairs received full to partial credit, but remember bacteria typically have about 25% of each base.

4. Amino acid #200 in the E. coli Tryptophan synthase gene (trpA) is lysine. A trpA- mutant with no activity at all was found. Revertants were screened for growth in the absence of tryptophan and in addition to those with the normal sequence, other revertants were found to have ser, gln or glu at position 200.
a) Provide a model, with codons, that explains the original mutation and array of revertants, assuming:

1) the reversions result from intragenic mutation

original mutation AAA/G to UAA/G, a stop codon truncating the protein with no activity

The revertants could be UAA/G back to AAA/G (lys); UAA to UCA (ser); UAA/G to CAA/G (gln) and UAA/G to GAA/G (glu) all of which are single base changes

2) intergenic mutations

These would be nonsense suppressor mutation changing the anticodon to pair with UAA/G. In each case the tRNA could change to something like AUU

b) List other potential amino acid substitutions that could occur and prospective reasons they were not seen in the original revertant screens.

Tyr, glu leu and also trp if the original was UAG

It is possible that these substitutions would not allow proper folding of the protein to regain activity or that the loss of a functional tRNA for one of these amino acids would not allow growth to continue.

5. Recalling that oxidatively deamination of C produces U and of 5MeC produces T

a) What is/are typical causes of oxidatively deamination?

NO\textsuperscript{2-} and free radicals

b) Assuming the change occurs in a double strand of DNA, describe at least 2 mechanisms that may be used to correct the potential damage.

Excision repair where a ‘chunk’ of bases surrounding the site is removed and replaces with DNA polymerase and ligase

Glycosylases that remove the base from the backbone triggering replacement or base excision repair

c) It has been held for a long time that yeast and Drosophila do not have 5MeC in their DNA (If they do it is very rare compared to mammals, plants, etc.) What repair model(s) would be affected if 5MeC is not a factor.

Any repair soon after replication where methylation identifies the old strand so that the new will be corrected to match it, rather than vice versa.
6. Caffeine is ‘negative’ in the Ames test but is sometimes found to promote or decrease mutagenicity of other compounds when tested together in CHO tissue culture cells or more complex systems.

   a) What is the Ames test? How does it work?
   A test for detecting mutagens. His- mutants of Salmonella are plated on minimal medium in the presence of the prospective mutagen; if a higher than background number of colonies is found the agent is a mutagen. Different strains have different initial mutations so that the mutagens ability to correct transitions, transversions and frameshifts define the types of changes mutagens cause.

   b) How can the different results be explained?
   Typically, it has been explained that some repair mechanisms are more error prone than others. If caffeine somehow interferes with a very efficient system, more mutations will be found and vice versa.

7. Apolipin protein B in humans comes in two forms; ApoB-100 that is 4,536 amino acids long that is made in the liver and ApoB-48 that is 2,152 amino acids long which is made in the small intestine. The first 2152 amino acids of the long form are the same as the short form, with amino acid 2153 in the long form being Gln.

   a) Provide two plausible explanations for how the two forms of Apolipin B protein are made from the same gene.
   1) site specific mRNA editing in the intestine changes CAG to UAG (this is the actual case)
   2) alternate splicing where the 3’ exons are all removed
   3) post translational modification where a protease cleaves off the carboxy terminus.

   b) Suggest a kind of data that would convince you that both forms come from the very same APOB gene.
   Detecting individuals with mutations in the first 2152 amino acids and showing both forms are altered. (Part credit for just sequencing, but there could be duplicate genes)

8. Donor DNA from a Str⁺, his⁺, lac⁺ strain was used in a transformation experiment with a recipient that was Str⁻, his⁻, lac⁻. 3% of Str⁺ colonies selected on rich medium were Lac⁺ when replica tested for growth using lactose, but none were his⁺. However all of the colonies that grew when selected first on lactose minimal medium were also his⁺. Which genes are linked. What error did the experimenter make?

   Str and lac for sure. When he tested for ability to use lactose, it was on minimal medium; therefore all colonies that grew also had to be his⁺, so this was not a valid test. He should have tested on lactose + histidine, then replicated onto minimal to ID his⁻ and his⁺
9. In the lecture material we covered the fact that the amino acids ile and val are made by enzymes in the same pathway, as shown in the cartoon below:

```
pyruvate  B   C   valine
E1  E2  E3
αKetoglu. B'   C'   isoleucine
 E4  E5
D   leucine
```

In plants, leucine is made from C' in a two-step reaction as indicated.

a) Which of the amino acids would need to be added to allow mutants lacking each of the enzymes to grow
   E1  val ile & leu
   E2  val ile & leu
   E3  val and ile
   E4  leu

b) Which intermediates alone or together would be the least necessary to allow mutants lacking the following enzymes to grow?
   E2  C and C'
   E3  none, only val and ile
   E4  D

c) E1 (ALS) is the target for many herbicides and in a relatively few years, many weeds have developed resistance that generally can be traced to the ALS gene itself. 1) What does this observation suggest?

   The change alters the enzyme such that the ALS herbicide no longer inhibits while still allowing it to function

   2) Do you think these ALS herbicides pose a problem to humans? (Explain)

   Not likely: we get these amino acids in our diets (they are ‘essential’ meaning we can’t make them

10. a) Fill in the chart below telling whether β-galactosidase and permease would be found at high (H), Low (L) levels or absent (A) in each strain for the conditions shown. FS indicates the site of a frameshif mutation. Partial diploids are shown with the genotypes of each component.
### Table

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inducer absent</th>
<th>Inducer present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-gal</td>
<td>perm</td>
</tr>
<tr>
<td>1.</td>
<td>P I P^− O Z Y A</td>
<td>A</td>
</tr>
<tr>
<td>2.</td>
<td>P^− I P O Z Y^− A</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>P I P O Z^− Y A</td>
<td>A</td>
</tr>
<tr>
<td>3.</td>
<td>P I P O Z^{FS} Y A</td>
<td>A</td>
</tr>
<tr>
<td>4.</td>
<td>P I P O Z^− Y A</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>P I P O^− Z Y^− A</td>
<td>H</td>
</tr>
<tr>
<td>5.</td>
<td>P I S P O Z Y A</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>P I P O^− Z Y^− A</td>
<td>H</td>
</tr>
</tbody>
</table>

b) Mutants of the lacI gene have been identified that prevent the formation of active (Operator-binding) tetramers with themselves or with normal repressor transcripts. Predict the consequences on regulation of these mutations when heterozygous in a partial diploid.

This would greatly lower the amount of functional repressor likely making the strain at least partially constitutive.

### Prediction

**c** Predict the consequences of a defect in the gene that encodes the CAP protein.

**If there is no catabolite activator protein to bind the cAMP when times are tough, it would still not be possible to activate the lac operon, even if lactose is present.**

11. Short answer questions:

**a)** What is the principle of mapping via transduction?

**Only genes that are very close together will be transferred in a single virus infection event.**

**b)** How does a eukaryotic mRNA differ from one in a prokaryote that makes the same protein? **The eukaryotic mRNA has a 5’CAP and a poly A tail. Prokaryote mRNA also has a Shine Delgrano motif (AGGA) in the leader for ribosome attachment. Saying it also has had introns removed etc. is fine.**

**c)** Neither selenocysteine or hydroxyproline show up on the genetic code sheet, yet both are found in proteins. **How do they get there?**

**Hydroxyproline is a post translation modification of a prline. Selenocysteins is imnorporated at UGA codons from a specific tRNA where the Se is added to a cys and requires a context recognized by a SECIS sequence.**

**d)** Give two mechanisms that account for the function of antibiotics.

**Prevent cell wall synthesis (penicillin)**

**Inhibit translation on bacterial-sized ribosomes (the mycins)**
e) Most signal peptides are cleaved as a protein ‘reaches its destination’, but the nuclear signal in eukaryotes is not. Why not?

The nuclear membrane ‘disolves’ during each mitosis so those proteins need to be able to get back in.

f) Name a non-radioactive active element that was used to verify a genetic concept and also tell what concept that was.

N15 was used to demonstrate that DNA replication is semi-conservative

12. More short answers

a) Give two lines of evidence that showed prokaryotic 'chromosomes’ are circular.

1. Autoreadiographs from H3-labeled DNA present during replication

2. Conjugation where no matter where started, if allowed to continue to completion, the whole chromosome was transferred

b) What do telomerase, ‘SRP” and snRNPs’ do/or stand for and what do they have in common.

Telomerase is a ribo-enzyme for restoring the termni of linear chromosomes following replication

SRP = signal receptor particle for trans-membrane translation of proteins into the ER based on a signal peptide at the beginning of the protein being made

snRNPS are small nucleo-ribo-proteins used to splice out introns from pre-MRNA

All have an RNA component

c) How do we know that DNA polymerases have a 3’ to 5’ editing function?

Mutations that eliminate the editing component can increase the number of mis-incorporated bases X 10,00

d) How has Beadle and Tatum’s “one-gene : one enzyme” concept been adapted to modern knowledge?

First, one gene-one polypeptide to account for subunit enzymes, then one gene several pp’s to account or splicing, then the recognition that RNAs could also be catalytic, etc.