1. The small *B. subtilus* phage SP8 is unusual in that one of its DNA strands is very purine rich, meaning the other is pyrimidine rich. A 1963 experiment showed that SP8 mRNA was complimentary only to the pyrimidine rich strand, which is denser than the purine rich strand.

   A) What bases occurred most often in each strand?

   Purine rich: **Adenine and Guanine**
   Pyrimidine rich: **Cytosine and Thymine**

   B) Suggest a method for separating and purifying the two strands.

   **Heat to separate; use density gradient centrifugation to purify the two strands**

   C) Although the original procedure did not use isotopes at any step, it might be advantageous with today’s tools to do so. What could be labeled by growing the host and SP8 with the following:

   - α-^P32^-labeled UTP (α is the P closest to the ribose) **RNA**
   - H^3^-labeled deoxynucleoside-triphosphate **DNA**
   - S^35^-labeled methionine **proteins**
   - N^15^-rich nitrogen **DNA, RNA, protein**

   Which, if any of the above would be useful in showing that SP8 mRNA is complementary only to the pyrimidine-rich strand of DNA? What is the process that would be used?

   **Perhaps the P^32^-labeled RNA and/or H^3^-labeled DNA. Although both labels would occur in both the purine and pyrimidine rich strands, the level of dual-labeled (or just labeled mRNA) that re-annells to the pyrimidine rich DNA strand should be significantly greater than that annealing to the purine rich strands.**

2. In a word or two, what is the role of the following components in DNA replication?

   - **pol I** replace RNA primers in Okazaki fragments of lagging strand
   - **pol III** primary extension of both strands
   - **SSB protein** protect single strands from nucleases and keep separated
   - **Topo-isomerase** relieve supercoiling
   - **DNA ligase** connect Okazaki fragments together
   - **Helicase** unwind DNA double helix
   - **Primase** create RNA primers on lagging strand

3. *rip1* mutants of Arabidopsis can be maintained in heterozygous plants, that are pale yellow and dwarf; homozygous plants are very small, grow only on supplemented medium and are more like a callus than a plant. The mutants have been found to have numerous non- or dysfunctional plastid and mitochondrial proteins. Based on just this information, propose a hypothesis that could explain how a single nuclear gene defect could cause the problems described and propose a way to test your hypothesis.

   **These mutants actually interfere with the mRNA editing which would have been a good hypothesis since it occurs at a high level in both organelles. Sequence comparisons of the organelle mRNA (or the defective proteins) in mutant versus normal would provide a test.**

   Another ‘likely’ culprit could be a faulty signal receptor for transfer of proteins into the organelles; tracking labeled proteins should help. Several other possibilities were mentioned and received partial credit.
4. In the 1960's Yanofsky and his students were able to determine the entire 268 amino acid sequence of Tryptohan Synthase subunit A from *E. coli* which which enabled multiple studies to establish the effects of specific trpA mutations. Following is a ‘reconstructed example’ of how the mutants could be used. A series of mutants selected following acridine mutagenesis had no enzyme activity. In some cases, a second treatment restored activity, but often at less than 100% activity levels. The original sequence of the only fragment showing a change between normal and the restored enzyme and a functional double mutant are shown below

original: **ile•ile•glu•gln•his•ile•asn•glu•phe•glu**  
Restored: **ile•ile•ser•asn•ile•leu•met•ser•phe•glu**  

a) predict the effects of the first and second treatments with acridine, in principle or exactly, if possible. Acridine most often induces single base-pair insertions or deletions. Here, taking away the first G in the first glu codon and inserting a U (T in the DNA sense strand) at the second glu would restore the framshift to give the sequence shown. The mutations could occur in either order since no stop codon is created in the out-of-normal-reading frame section.  
b) as far as possible, what codons are used for each of the amino acids in the wild type strain.  

For the segment **glu•gln•his•ile•asn•glu**  
GAG CAA CAU AAU AAU GA

5. Which amino acids are most likely to be inserted by a nonsense suppressor mutation for UAG. Any tRNA with an anticodon that is 1 base different: Gln, Trp, could occur by a transition while Tyr, Lys, Glu, Ser and Leu would require single base pair transversions.

6. A series of mutations affecting different genes(*pur1*to *pur5*) in Neurospora were found that required both purines to grow, while others could be classified as either Adenine auxotrophs (*ad-8* and *ad-4*) or Guanine auxotrophs (*gua1* and *gua 3*) in that they could be grown on A or G, respectively in strains where a recycling gene called HGPRTase that can attach a ribose–phosphate to the base is still functional. Shown below are a series of ‘feeding tests with potential precursors that helped to establish the pathway. It is also significant that double *ad 4* and *gua1* auxotrophs were found to accumulate inosine-5’phosphate so long as no *pur* mutations were present.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Growth (+) on minimal medium supplemented with compound(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td><em>pur1,2,4</em></td>
<td>+</td>
</tr>
<tr>
<td><em>pur3</em></td>
<td>+</td>
</tr>
<tr>
<td><em>pur5</em></td>
<td>-</td>
</tr>
<tr>
<td><em>ad8</em></td>
<td>-</td>
</tr>
<tr>
<td><em>ad4</em></td>
<td>-</td>
</tr>
<tr>
<td><em>gua1</em></td>
<td>-</td>
</tr>
<tr>
<td><em>gua3</em></td>
<td>-</td>
</tr>
</tbody>
</table>

*(G and A are the bases guanine and adenine; tests made in HGPRTase positive strains)*
a) design a potential pathway for biosynthesis of purines. Hint: make separate paths involving the pur, ad and gua mutants and then combine them.

\[
\text{pur}1,2,4 \xrightarrow{S} \text{pur}3 \xrightarrow{C} \text{lp?} \xrightarrow{Y} \text{ad8} \xrightarrow{A} \]  
\[
\text{gua1} \xrightarrow{G} \xrightarrow{X} \text{gua3} \]  

b) What does the fact that three independent genes pur1, 2 and 4 all show the same responses on all the compounds suggest? (give two possibilities)

1) They code for 3 peptides that function in the same enzymatic step  
2) There are 'missing' intermediates before S

c) I (inosine, which is the base hypoxanthine attached to ribose) was included in the tests because it has a structure resembling a potential purine precursor, but I did not support growth of any mutants. What does the fact that I-5'-phosphate accumulates in ad and gua double mutants suggest? That I-5'P is an intermediate, and perhaps phosphorylated compounds are the true intermediates; it also suggests that the 'essential' HPRTase can add the phosphate to Y, X, A and G nucleosides, but not to I.

7. In a series of bacterial transformation experiment involving the genes man (ability to use mannitol as a carbon source), aroD (require aromatic amino acids to grow and rifR (rifampicin resistance which is associated with an altered ribosomal protein) the following results were obtained:

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Selected marker</th>
<th>Unselected marker</th>
<th>% 'co'</th>
</tr>
</thead>
<tbody>
<tr>
<td>man, aroD+</td>
<td>man+, aroD</td>
<td>aroD+</td>
<td>man</td>
<td>5</td>
</tr>
<tr>
<td>man+, rifR</td>
<td>man, rifS</td>
<td>man+</td>
<td>rifR</td>
<td>26</td>
</tr>
<tr>
<td>rifS, aroD+</td>
<td>rifS, aroD</td>
<td>aroD+</td>
<td>rifS</td>
<td>54</td>
</tr>
</tbody>
</table>

A) make a ‘relative order’ map of the 3 genes.  
aroD   rif    man

B) For each pair, give the media components that would be used for doing the selection for the first marker and then tell how the presence or absence of the previously ‘unselected’ marker would be tested.

man, aroD: Select for aroD+ on glucose-minimal; replica plate onto mannitol minimal and identify the % of colonies that do not grow (aroD+ and man co-transformed from donor) 

man+, rifR :grow on mannitol minimal: replicate onto same with rifampicin; % mannitol+ that grow are co-transformed 

rifS, aroD+: Select for aroD+ on glucose-minimal (no aromatic amino acids); replicate onto medium with rifampicin and identify % of aroD+ colonies that do not grow.
8. Chemotherapy for cancer often involves a combination of compounds including base analogs such as 6-thioguanine, along with methotrexate, a drug that prevents de novo synthesis of folate and consequently prevents purine biosynthesis. What is the rationale for using these or related compounds? Base analogs are mutagenic when incorporated into replicating DNA, which makes cancer cells susceptible. Blocking the synthesis of purines with methotrexate reduces the pools of genuine bases, making incorporation of the analog more likely. Lethal or reduced growth mutations slow growth of the cancer.

9. a) Tell whether synthesis of the enzymes β-galactosidase, permease and transacetylase would be absent (A) regulated (R) or constitutive in the following strains of E. coli. Assume any mutant (-) allele is a completely inactive mutation for that component while those with a superscriptFS result from a frameshift mutation.

<table>
<thead>
<tr>
<th>E. coli strain- lac operon genotype or genotypes</th>
<th>β-gal'ase</th>
<th>permease</th>
<th>TA'sae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. P I P O Z YFS A</td>
<td>R</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>2. P I P O Z Y A</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>3. P I P O - Z - Y A</td>
<td>A</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>5. P I P O ZFS Y A / P I P O - Z - Y A</td>
<td>A</td>
<td>C</td>
<td>A</td>
</tr>
</tbody>
</table>

b) Which strains, if any would differ in apparent regulation response if lactose rather than IPTG were used as the inducer? Explain why. Strain 1 is regulated for β-gal’ase but has a frameshift disrupting the Y gene; if no lactose could get in, there would be no allolactose so β-gal’ase would not be made even in lactose. c) What effect on regulation of the lac operon would be predicted from the following?

1) A deletion of the CAP site: Lower levels of induction that expected when glucose is limiting
2) A mutation in the gene that encodes cAMP phospodiesterase, the enzyme that converts cAMP back to AMP: High levels of cAMP would allow induction of the lac operon even when glucose is present
3) Adding dibutyryl cAMP, an analog of cAMP that diffuses directly into E. coli cells: This would also ‘trick’ the system into induction even when glucose is present.
4) What allows strains 4-6 in the table to have two copies of the lac operon per cell? The lac operon is part of an F’ so can be introduced by mating to an F- female.

10. A number of studies have looked at the effects of caffeine on UV mutagenesis in different organisms. Predict the consequences of the scenarios below if the same effect happens in humans, or in the last case on the risk of cancer in humans.

a) In S. pombe, a type of yeast, caffeine overrides the normal signal to proceed from the G2 phase of the cell cycle into mitosis. This would allow mitosis to begin before DNA repair has finished so that pre-mutational lesions (in skin from UV, but also in other cells from other mutagens) would become fixed in the DNA.
b) In *E. coli*, caffeine inhibits the binding of photolyase to Thymine dimers. If humans had photolyase, less repair would occur in skin cells, likely causing a much higher rate of skin cancer.

c) In *E. coli*, caffeine allows non-specific DNA binding of the UVR-A component of the dark repair system for excising T-T dimers. This could lead to failure to repair true T-T dimmers and increase the 'repair' at non-mutant sites, likely also increasing the odds for a mistake. Unless the backup repair was more efficient, more skin cancers would be predicted.

d) In human keratinocytes, caffeine promotes the level of apoptosis of damaged cells. If damaged cells are more likely to 'die', there should be a decrease in the number of cases where cell division becomes uncontrolled.

11. a) Nitrous acid can oxidatively deaminate C in DNA or RNA, changing it to U. What amino acid substitutions could a single base change have on a gene or mRNA encoding a CCC codon? CCC to UCC gives serine; CCC to CUC gives leucine

b) Would the changes in mRNA be considered mutations? (why or why not).

No; those changes would not be inherited, so are not considered to be mutations.

c) Oxidative deamination of A creates Hypoxanthine (H pairs with C) and of G creates Xanthine (X pairs with C). Assuming the original mutations in part a eliminate function, what amino acid(s), could be present in potential revertants of the mutations following a second treatment with nitrous acid?

From the UCC serine codon (TCC/AGG) in the DNA, A to H would end up as CCC in the codon to give proline. C to U in the middle base would give UUC or phe. The end change would give serine, but that is nonfunctional. From the CUC (Leu) codon, C to U would give phe, A to H would restore CCC for proline and the last C to U would give leu which is nonfunctional.

12. At least 2 amino acids have been shown to be incorporated during translation of mRNA that are not listed on the code sheet. How does this happen?

Both pyrolysine and selenocysteine are enzymatically modified when lysine or cysteine, respectively, are attached to special tRNAs with an anticodon that recognizes a stop codon that is 'in context' in key mRNAs. Another protein competes with the release factor, allowing the modified amino acid to be inserted during translation.

13. Very short answers:

a) What types of pathways can use attenuation for regulation?

Amino acid biosynthesis in prokaryotes (stalling at codons for that amino acid in a leader sequence alters the conformation of the nascent mRNA allowing continued transcription and translation).

b) How do the 5’ and 3’ ends of eukaryotic mRNA differ from those in prokaryotes?

Eukaryotic messages have a 5’ CAP and do not have a Shine Delgarno sequence, and the 3’ end has a poly A tail added.

c) What is the minimum number of tRNAs needed for arginine? 3

d) What do snRNPs do? Bind at splice junction to facilitate removal of introns.

e) What does the Ames test test? Mutagenicity of compounds (and shows whether they can cause transition and/or transversion and/or frameshifts)
f) What is the difference between generalized and specialized transduction? Generalized transduction occurs when random fragments of a donor genome can be packaged into a phage head and transferred to a recipient. In specialized transduction, only host DNA that flank the integration site of a temperate phage can be transferred.