1) In the experiment that showed the *E. coli* chromosome is circular, the experimentors used tritium-labeled thymidine (TdR) for producing autoradiographs.

a) Why did they choose thymidine over other possible labels?

*Thymine is only incorporated into DNA (TdR the source of the T nucleotide)*

b) Why tritium \(^{3}\text{H}\) rather than \(^{14}\text{C}\) or \(^{32}\text{P}\) or \(^{15}\text{N}\), all of which are present in thymidine?

a) It is a weak ‘emitter’ that won’t lead to overexposure of the film that would obscure the position of the DNA under high power magnification

b) It has several H’s that can be replaced by tritium so that the pure compound can be labeled ‘in vitro’.

2) What are the sources of energy involved in translating mRNA and what ‘steps’ are involved in using that energy?

ATP to AMP + PPi is used to attach each amino acid to its tRNA with a high energy bond

GTPs are used to a) load charged tRNA onto ribosome and b) ratchet the mRNA and tRNAs from A site to P site.

3) Initiation of transcription and translation of mRNA in eu- and prokaryotes are similar but not exactly the same. What differences would be critical if you wanted to express a eukaryotic gene in *E. coli*?

The TATA box and other key promoter elements are at different relative locations for transcription (TC) initiation; eukaryotic TC often needs several response elements bound to distal enhancer elements to initiate; these would not work in *E. coli*.

For translation initiation, eukaryotes use a cap-binding protein and several other eIFs to locate the 5’ end of the mRNA and to get the mRNA associated with the small ribosome subunit. In *E. coli*, it is essential to have a Shine Delgarno sequence built into the leader to do this.

4) An enzyme in humans and other animals converts a number of glutamic acid residues in proteins involved in blood clotting to \(\gamma\)-carboxyglutamate; if the conversion is not made, the clotting factors are non-functional. The enzyme that makes the conversions, \(\gamma\)-glutamyl carboxylase (GGCX), has sequences that span membranes of the endoplasmic reticulum and Golgi. It is the product of a 13 kb gene with 15 exons and although an alternatively spliced mRNA lacking exon 2 is frequently present, only a 758 residue polypeptide, as modified by glycosylation has been isolated. The active site for binding Vitamin K, a substrate for the conversion reaction, is located inside the lumen of the ER. Based on this information, list as many different ‘reasons’ as possible in addition to simply causing misfolding as a result of a
missense mutation, where a single base substitution in the gene could lead to the lack of functional GGCX.

Possibilities include:
1) a change in a splice site signal
2) a change in any transmembrane spanning amino acid sequence
3) a change to a glycosylation signal
4) a change to the active site and or VitK binding site
5) a change in the signal peptide so that it does not bind the SRP
6) creation of a nonsense codon
7) an alteration in a key promoter element
8) a change of the start codon or the start codon "context" bases
9) loss of a poly A tail signal
10) new modification site created that is detrimental
11) rare codons created that slow translation and lead to alternate folding
12) etc.

5) In *E. coli*, amber suppressors, (amber is an early name for UAG codons) have been found that insert, for example, serine, leucine, or tyrosine only for UAG. Suppressors of UAA (ochre) typically also suppress UAG mutations.

a) What is the basic explanation of these suppressor mutations?

A single base change in the anticodon of a tRNA that carries one of these amino acids makes it pair with UAG

b) Why do ochre suppressors often also suppress amber mutations and not *vice versa*?

‘Wobble” They would have a U in the 3rd pairing position and it can pair with A or G

c) Can you predict other amino acids that might be inserted as a result of similar amber suppressor mutations?

Trp, Gln, LYS and GLU could all have anticodons that could bind to UAG with a one base change

d) Not all amber suppressors are effective on some amber mutations as they are on others. Why?

Each inserts a different amino acid; some of these may not permit proper folding. Less likely but possible, some may be less effective in competing with a release factor or the loss of one tRNA gene copy may be more detrimental for some amino acids than others.
6) Match the letters for the activities in the following lists to their function or functions/activities (if any) in the second list:
A-Polymerase I, B-Polymerase III, C-Helicase, D-Primase, E-Ligase, F-SSB protein, G-gyrase

<p>| | | | | | | |</p>
<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>C</td>
<td>Unwind DNA double helix</td>
<td>F</td>
<td>Maintain unwound strands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>Insert RNA bases</td>
<td>A</td>
<td>remove RNA bases</td>
<td>A, B</td>
<td>5’ to 3’ polymerase</td>
</tr>
<tr>
<td>A, B</td>
<td>A, B</td>
<td>3’ to 5’ exonuclease</td>
<td>E</td>
<td>connect 3’ OH to 5’ PO4</td>
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<td></td>
</tr>
</tbody>
</table>

(G is a topoisomerase but not required) | relieve supercoiling | E | connect 3’ OH to 5’ PO4 |

7) Multiple essential compounds often share a common precursor. Assuming all compounds in the table below can enter and function in Neurospora, develop a pathway that will account for the growth responses for the 4 mutants. Show where each mutant is blocked on your pathway.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>aux1</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>aux2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>aux3</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>aux4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

8) Eukaryotic structural genes are often much longer than need be to account for the functional polypeptide encoded. List at least 3 reasons that help account for this fact.

1) introns, 2) signal peptides that are removed, 3) pre- and pro forms are reduced enzymatically 4) long upstream promoter elements, 5) include 3’ untranslated coding sequences. (note poly A tails are not in the gene)

9) A) Which amino acids have codons where a single base substitution can create a samesense (synonymous) mutation. For each answer tell whether the change is a transition, (I); transversion, (V); or both (B) for the following situations:

1) the mutation is in the first base?

Leu U to C = transition , Arg, C to A = transversion
2) the mutation affects the middle base?

No sense codons, only UAA $\rightarrow$ UGA transitions could qualify

3) the mutation affects the third base?

All with 2 codons = transitions, all with 4 = both, ile with 3 = both

Only Met and Trp with single codons would not be listed

9-B) Is there any way such a change could alter gene function? Explain.

10) Normal LexA protein in *E. coli* binds CGAACNNNNGTTCG sequences in the promoter of 17 different 'SOS' genes, including its own. When severe DNA damage occurs, a protein called recA cleaves LexA so that in can no longer bind that sequence. Predict the consequences of:

1) a mutation that changes only the recA target site on LexA.
   This would mean the Lex protein would still bind the promoter site of all SOS genes even if DNA damage is severe, preventing the damage override from occurring; expect even more mutations, cancer or cell death

2) one G to A change in the LexA binding site in:
   a) its own promoter
   Lex A production would occur with or without cell damage and could prevent SOS rxn
   b) one of the other SOS promoters
   That gene would become constitutively expressed; can’t predict direct consequences

11) A series of *E coli* strains, all with the same set of selectable markers but with different Hfr insertions were mated to the same female which was negative for all the markers. In each cross the matings were halted after 30 minutes, and donor markers present in the exconjugant females were determined by a series of replica plates. The results are shown below. Note that in each case the markers are listed in alphabetical order, since no time-of-entry analysis was conducted.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Donor f, Markers Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFR-1</td>
<td>I, S, T</td>
</tr>
<tr>
<td>HFR-2</td>
<td>E, F, R</td>
</tr>
<tr>
<td>HFR-3</td>
<td>N, O, S, W</td>
</tr>
<tr>
<td>HFR-4</td>
<td>F, I, L, U</td>
</tr>
</tbody>
</table>
HFR-5 D, E, N, O
HFR-6 F, R, U

A) Suggest a method for eliminating the HFR donors from ‘contaminating’ the results.

Male specific phages would be good since the ‘maleness’ of an Hfr would not be transferred within until 90-100 minutes of mating. It would take several different antibiotic resistance selectable markers scattered around the chromosome to use that method, and the knowledge of when each would be transferred to be effective.

B) Draw a map showing as far as possible the locations of the genes and HFR insertion sites including a transfer direction consistent with your map.

(Arrows can be at either end of group)

12) An HfrB7 donor transferred genes malT (maltose utilization) str (streptomycin resistance) and argC (arginine auxotrophy) all very close to 60 minutes of transfer into a malT\textsuperscript{+}, str\textsuperscript{s}, argC recipient.

a) Suggest a method, other than conjugation with a different Hfr, for determining the actual order of these genes.

Since the 3 genes are so close together, Transduction with phage P1 would work or just transformation.
b) What media could be used to define the genotypes with respect to these three genes of colonies that were first identified on glucose complete medium? (ie establish a set of media for use in replica plating).

**Glucose minimal would identify His\(^+\) colonies**  
**Glucose + His + streptomycin would ID Strep\(^R\) colonies**  
**Maltose medium with histidine would identify malT\(^+\) strains**

c) Describe the data you would expect from the replica plating results if the str gene is between the other two but closer to malT.

More malT\(^+\) colonies should also be strep resistant than his\(^+\).  

13) Tell whether synthesis of β-galactosidase, permease and trans acetylase would be absent (A) regulated(R) or constitutive in the following strains of *E. coli*. Assume a negative allele is completely inactive; superscript\(^{FS}\) indicates 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’ase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. P I P O Z Y(^{FS}) A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. P(^{+}) I P O Z Y A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. P I P O(^{+}) Z(^{-}) Y A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. P I P O(^{+}) Z(^{-}) Y A / P I P O Z Y(^{-}) A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. P I P O Z(^{FS}) Y A / P I P O(^{+}) Z(^{-}) Y A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. P I P O Z(^{-}) Y A / P I P(^{-}) O(^{+}) Z(^{-}) Y A</td>
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<td></td>
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</tr>
</tbody>
</table>

Which, strains, if any would differ in apparent regulation response if lactose rather than IPTG were used as the inducer?

#1 – if lactose doesn’t enter would appear to be lacZ A not, R  
#6 without any b-galactosidase the permease and TAase would appear to be negatives