Biology 50-384 (Microbiology): Exam #2

1. You have isolated mutants in the following genes in the motile bacterium E. coli. These mutations result in the formation of a nonfunctional gene product. Based on your knowledge of chemotaxis, what would be the effect of each mutation (be specific) on movement and chemotaxis.

- **cheY (2.5 points):** When phosphorylated, CheY interacts with the flagellar motor which results in ccw rotation and thus tumbling. A CheY mutant would never be able to do this and thus the flagella would predominantly rotate cw resulting in running in one direction with little or no tumbling. Thus, since CheY mutants can’t change direction, they do not exhibit chemotaxis.

- **cheA (2.5 points):** CheA phosphorylates CheY as described above. Thus, the mutant behaves as described above. CheA also phosphorylates CheB which then demethylates MCP. This CheA mutant would have an increase in methylated MCP which would normally result in a more active CheA. However, since there is no CheA, this does not matter.

- **cheZ (2.5 points):** CheZ dephosphorylates CheY which results in the cessation of tumbling. Thus, in a CheZ mutation, CheY can never be dephosphorylated and so the organisms always tumbles. Because the organism can not run, it does not exhibit chemotaxis.

- **MCP (2.5 points):** This question was a bit complex since I did not specify if all MCPs types were nonfunctional or just one particular MCP type. Also, to add to the complexity, there are multiple domains on the protein that can be mutated as some of you answered with respect to a particular domain mutation (i.e. only the signal transduction domain between MCP and CheA was nonfunctional). I’ve provided a couple of possibilities below. Please see me if you think that your answer marked incorrect is correct according to your interpretation of the question

    If all the MCPs types in the cell were mutated, CheA would never be autophosphorylated and the cell would thus have only (or mostly) runs as described above for a CheA mutant. The organisms would not be chemotactic.

    If only one type of MCP was mutated then the cell would not respond to that chemoattractant because the chemoattractant could not bind. However, the cell would still run and tumble because other MCP types would interact with CheA initiate the signal transduction pathway for tumbling.
2. You are studying transport of a synthetic compound (compound X) into a bacterium. Based on the following experiments and what you know about transport of compounds into a bacterial cell, propose a mechanism for how the compound enters the cell, listing components in the transport system (5 pts) and any energy requirements (5 pts) and the type of transport (5 pts).

Exp. 1: You measure the rate of uptake of compound X into the cell as a function of concentration and obtain the following data:

Exp. 2: You disrupt the PMF of the cell membrane using a poison and find that transport of compound X no longer occurs.

Exp. 3: You obtain mutants that do not transport compound X. In addition, these mutants show an increase in periplasmic Na⁺ concentrations relative to non-mutants.

(15 points total – see above)
Exp #1 shows that X does not enter the cell by simple diffusion (see Figure 5-1 in text). Exp #2 suggests that the PMF is the energy source for transport of X. Thus, transport does not occur by facilitated diffusion. Exp #3 suggests that both X and Na are transported into the cell using the same carrier. If there is a mutation in this carrier then no X will enter the cell and less Na⁺ will enter the cell and so it will build up in the periplasm. There are probably other ways for Na⁺ to enter the cell as it is cotransported with other compounds. Taken together, the following model can be proposed: Transport of X occurs via active transport using a symport protein that transports both X and Na⁺ into the cell, down the Na⁺ gradient. The Na⁺ gradient is generated via a Na⁺ / proton antiport system, down the proton gradient. Finally, the proton gradient is part of the PMF and is generated by energy from the redox reactions in electron transport. Thus, the transport of X is via secondary active transport.

There were other possible answers that were also acceptable. One could have concluded from Exp #2 that the PMF was required to generate a large amount of ATP need for transport of X, and that the transporter hydrolyzed ATP to transport in both Na⁺ and X. This would be active transport mediated by ATP hydrolysis
3. The TCA cycle is widely distributed among microbes. In fact, organisms that do not use the TCA cycle to generate energy still have parts, if not all, of the cycle.

Why? (5 pts) The TCA cycle provides carbon skeletons for biosynthesis.

Name and briefly describe a type of catabolic lifestyle that does not use TCA as an energy source. (5 points): There were many answers possible – some are given here. Explanations are in lecture notes. Fermentation of pyruvate to lactate; oxidation of inorganic compounds; photosynthesis

4. You have isolated compounds that appear to disrupt catabolism of glucose in the bacterium E. coli. E. coli can obtain energy from either respiration or fermentation when grown in glucose medium.

<table>
<thead>
<tr>
<th>compound</th>
<th>effect after addition to E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>?ATP</td>
</tr>
<tr>
<td>#2</td>
<td>?ATP</td>
</tr>
<tr>
<td>#3</td>
<td>?ATP periplasmic pH = cytoplasmic pH</td>
</tr>
</tbody>
</table>

For each of the above compounds, propose what component of catabolism is affected. Why can the cells still grow with compounds #1 and #2, but not for #3 (15 pt total)?

Cpd #1: An increase in the levels of reduced CoQ suggest that CoQ is not able to donate its electrons to the next carrier in the ETC. Thus, electron transport will not proceed to the terminal electron acceptor and no oxidized CoQ will be available to accept anymore electrons. The decrease in ATP is due to the fact that ATP synthesis is NOT via oxidative phosphorylation now but is via substrate level phosphorylation only. This suggests that cpd #1 affects either CoQ (by not allowing it to release electrons) or the next carrier in the ETC (so that there would be nothing for CoQ to pass its electrons to). (4 pts)

Cpd #2: There could be many reasons why there would be a decrease in the level of ATP. The cpd #2 may have inhibited the activities of ATP synthase, pyruvate dehydrogenase which forms acetyl CoA for TCA cycle, a component of glycolysis or TCA cycle that decreases the rate of glucose catabolism, glucose transport that slowed down entry of glucose. (4 pts)

Cpd #3: The fact that the periplasmic pH = cytoplasmic pH indicates that there is no PMF. The most logical explanation for this is that the cpd #3 causes the membrane to become permeable to protons. Since the question only said propose what component of catabolism is affected, I also accepted the PMF as an answer. The answer that cpd #3 affects the proton pumps was partially correct, except that it would have to affect every mechanism of transporting protons out of the cell. Since there are many, this was unlikely. Thus, I took off 1 point. (4 pts)

The cells can still grow with cpds #1 and 2 because the cell may still derive enough energy from substrate level phosphorylation/fermentative pathways. The cells die with cpd #3 because the PMF is required for many essential cellular activities such as transport of nutrients.
5. All organisms need NAD(P)H. Why? (3 pts)

*NAD(P)H is required for biosynthesis.*

How do chemoorganoheterotrophs generate NAD(P)H? (3 pts)

*Glycolysis, TCA, pentose phosphate pathway*

Many chemolithotrophs have a unique problem in generating NAD(P)H. Based on your knowledge of the catabolic lifestyles of chemolithotrophs and of the chemistry of energy generation, explain what the problem is and how they solve it.

*Chemolithotrophs generally oxidize inorganic compounds as energy sources. The electrons from many of these compounds are at a less negative reduction potential (lower on the energy tower) than NADH/NAD+. Thus, they can not donate their electrons to NAD+ to generate NADH without the expenditure of energy. (2pts) They solve this problem with reverse electron transport in which the energy from the PMF (or from ATP) is used to drive the electrons to a compound with a more negative reduction potential (higher on the energy tower) (2 pts).*

6. The P/O ratio for oxidative phosphorylation from the oxidation of glucose is 3, while the P/O ratio for oxidative phosphorylation from the oxidation of inorganic compounds is closer to 1. Why is this? (5 pts)

*The electrons from the oxidation of inorganic compounds are at a less negative reduction potential (lower on the energy tower) than the NADH that is generated via the oxidation of glucose via the TCA cycle. Thus, as the electrons from inorganic compounds travel down the ETC (and down the energy tower) to the terminal electron acceptor, they release less energy (less protons are transported out) than the electrons from NADH. Since the H/O ratio is less and since P/O = H/O * P/H , the P/O ratio is less.*

What does it mean in terms of energy yields? (5 pts)

*Oxidation of inorganic compounds yields a lower amount of energy in the form of ATP than oxidation of glucose.*
7. Briefly explain how redox reactions are coupled to proton translocation (in other words how are the electron transport chain and the generation of PMF coupled mechanistically)? Any of the following three answers would have been accepted (10 pts).

Proton pumps: The energy from the redox reactions that comprise the ETC (transfer of electrons from one carrier to another = redox reaction) is used to pump protons to the periplasm generating a PMF.

Loops: In the ETC, hydrogen carriers and electron carriers are involved in redox reactions and alternate in the chain: 2 hydrogen are taken up by hydrogen carrier \( \rightarrow \) electrons are passed to electron carrier at the outer surface and protons are released to the periplasm \( \rightarrow \) electron carrier will pass 2 electrons to hydrogen carrier which will then pick up to 2 protons to make 2 hydrogen \( \rightarrow \)

Q cycles: reduced quinones carries 2 protons and 1 electron across the membrane releasing the protons to the periplasm \( \rightarrow \) becomes oxidized to semiquinone \( \rightarrow \) semiquinone picks up 2 more protons and the other electron to become reduced \( \rightarrow \) repeat

8. What is the primary molecule from which the following inorganic compounds are incorporated into organic material?

(3.3 pts each)

nitrogen \( \text{NH}_3 \)

sulfur \( \text{H}_2\text{S} \text{ or cysteine} \)

phosphate \( \text{PO}_4 \)

9. There are two main classes of organisms based on their ability to incorporate C into cellular material. What are they and what are the differences between them? Give one example of C incorporation for each class.

Autotrophs use \( \text{CO}_2 \) as their sole carbon source. (3 pts)

Heterotrophs use reduced, preformed organic molecules as their carbon source. (3 pts)

Example of C incorporation for autotrophs is via carbon fixation by the Calvin-Benson cycle. (2 pts)

Example of C incorporation for heterotrophs is via glycolysis, pentose phosphate pathway, gluconeogenesis. (2 pts)
10. Define anaplerosis, give an example of it, and explain why it is especially important in heterotrophs.

Anaplerosis is the replenishing of depleted TCA cycle intermediates. (5 pts)

Examples of anaplerosis are “CO₂ fixation” such as the addition of CO₂ to PEP to generate the TCA cycle intermediate oxaloacetate OR the glyoxalate cycle which is a modified TCA cycle that bypasses decarboxylation steps allowing acetyl CoA to be incorporated into oxaloacetate and other intermediates. (3 pts)

Anaplerosis is especially important in heterotrophs because many times they incorporate carbon for biosynthesis and generate energy using the same pathway (glycolysis and TCA cycle). (2 pts)

11. You have obtained some sediment from a local pond which is known to contain the following organisms:
- sulfate reducer- grows on propionate (propionic acid) at pH 5.5-7.5
- methanogen- grows on CO₂ and H₂ at pH 5.5-6.5
- lactic acid bacterium- homolactic fermenter, grows on glucose from pH 4.0-6.0
- mixed acid fermenter- grows on glucose at pH 5.0-6.5
- propionic acid bacterium - grows on succinate (succinic acid) at pH 5.0-7.5

You add medium (pH 6.5) containing only glucose, sulfate, and mineral sources to the sediment and incubate the sediment in an anaerobic bottle at room temperature. About two weeks later you do some tests on the contents of the bottle and observe metabolic products of all five organisms present. How can you explain this result in light of the fact that the original medium contained only glucose and sulfate? Use a diagram to clarify your answer.

The reason that metabolic products from all 5 organisms are seen is that the organism that grows first generates end products that other organisms need for growth and so on… (7 pts). The only organism that can grow initially is the mixed acid fermenter as it only needs glucose and pH 5-6.5. This organism produces H₂, CO₂, many acids including succinate as end products or byproducts. The lowered pH allows the lactic acid bacterium to ferment glucose and grow. The H₂, CO₂ are used by the methanogen for growth. The succinate is used by the propionic bacterium which makes propionate. The propionate is oxidized by the sulfate reducer (sulfate was provided in the medium). (3 pts)
12. Temperature sensitive mutants have been isolated in the various proteins that are involved in DNA replication in *Escherichia coli*. Temperature sensitive mutants in any of the subunits of DNA polymerase III or in the helicase called Rep exhibit an immediate cessation of DNA replication after the shift to the nonpermissive temperature (quick stop phenotype). In contrast, temperature sensitive mutants in DnaA or DNA ligase exhibit a delay in the cessation of DNA replication after the shift to the nonpermissive temperature (slow stop phenotype). Based on your knowledge the function of the wild type proteins in DNA replication, explain why each mutation gives the phenotype listed above.

**DNA polymerase III – quick stop**

*DNA pol III adds the nucleotides to the new strand. If it is inactivated, it can not do this and DNA replication immediately stops.*

**Rep helicase – quick stop**

*Rep helicase melts the double helix of the DNA template so that the replication enzymes have access to the DNA strand. If Rep is inactivated by high temperature, the strands will not be melted apart and DNA replication immediately stops.*

**DnaA – slow stop**

*DnaA binds to oriC (the DNA replication initiation site) to begin DNA replication. If DnaA is inactive and as long as DNA replication has already begun, replication will continue even if DnaA is not active – thus DNA replication does not immediately stop.*

**Ligase – slow stop**

*Ligase joins the Okasaki fragments of the lagging strand together. If ligase is inactive, DNA synthesis can still continue for a while. However, since the fragments will not be joined, DNA synthesis will eventually cease. Thus DNA replication does not immediately stop.*
13. Consider the following gene and its corresponding mRNA:

\[
\begin{align*}
\text{mRNA:} & \quad 5'\text{AUUGCAAAAGGUAGGUAGUAAUCGGGAUCGUAAUUUAGUCUAA3'} \\
\end{align*}
\]

a) Label the start of transcription and the promoter region on the gene.
   See arrow (1.5 pts) and line above (note that I also accepted promoter as just the TATA box) (1.5 pts)

b) What is the function of the promoter?
   The promoter is the sequence of DNA to which the sigma factor of RNA polymerase binds to initiate transcription. It signals the beginning of a gene. (3 pts)

c) Label the 5' and 3' ends of each strand of the gene.
   See above (3 pts)

d) Indicate the DNA strand that served as the template during transcription.
   See above (3 pts)

e) Show the anticodon in the tRNAs that would recognize the underlined codons shown in the mRNA above. Label your anticodons with the 5' and 3' ends.

\[
\begin{align*}
\text{UCG} \rightarrow & \quad 3'\text{AGC5'} \quad (1.5 \text{ pts}) \\
\text{AUC} \rightarrow & \quad 3'\text{UAG5'} \quad (1.5 \text{ pts})
\end{align*}
\]