NAME:	
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Biology 50-384 (Microbiology): Exam #1 Answer Key

1. Clearly explain in 2-3 sentences why prokaryotic cells usually are smaller than eukaryotic cells.

Assuming a spherical cell...Because volume increases by the cube of the radius and surface area increases by the square of the radius, the larger the cell the smaller the surface area to volume ratio (2 pts). Prokaryotic cells are smaller than eukaryotic cells because they require a <u>large</u> surface area to volume ratio to allow nutrients to easily diffuse from the plasma membrane throughout the cytoplasmic membrane and to allow wastes to quickly diffuse to the plasma membrane from throughout the cytoplasm (2 pt). Eukaryotic cells do not require this because they have organelles that transport nutrients and wastes efficiently without relying on diffusion (2 pt). I also will accept that since many metabolic activities are carried out at the level of the cytoplasmic membrane which thus requires a large surface area to volume ratio.

The bacterium *Epulopiscium fishelsoni* was identified as a prokaryote in 1993. However, the organism can grow to a whopping size of 80 μ m by 600 μ m. How has this organism adapted to have such a large size and still be a prokaryote?

The organism has adapted by increasing its effective surface area via extensive foldings of the plasma membrane and by having a long thin cell shape that decreases the distance that nutrients and wastes have to travels. (4 pt)

2. Imagine a planet where life evolved in a non-aqueous environment. Cells on this planet contain a hydrophobic environment. Based on your knowledge of the properties of plasma membranes, draw a possible structure of the plasma membrane of these cells and <u>briefly</u> discuss why such a membrane would be best suited to these organisms in this environment. *The plasma membrane is composed of a phospholipid bilayer. Phospholipids are amphipathic; they have a polar hydrophilic end and a nonpolar hydrophobic fatty acid end. This amphipathic property allows the formation of lipid bilayers in which the hydrophobic fatty acids associate with one another and exclude polar substances and the polar ends interact with either the aqueous cytoplasm or periplasm.*

On this hypothetical planet the lipid bilayer would form in reverse orientation of the above description since the hydrophilic polar ends would NOT want to be in contact with either the non-aqueous environment or the hydrophobic cytoplasm. Thus the hydrophilic polar ends would associate with one another and the nonpolar hydrophobic fatty acid ends would contact the environments (see figure below) (6 pt). I also would accept a lipid bilayer with no polar groups at all or joined by a polar group embedded in the layer

This membrane would be best suited to these organisms in this environment because it would be the most stable form based on the amphipathic properties of phosphospholipids. I also will accept an alternative interpretation of the question which was that this membrane would still retain important features of a plasma membrane which include retains the cytoplasm, semipermeability, transport of nutrients and wastes, and generation of energy (4 pt).

3. Based on your knowledge of the cell walls in gram positive and negative bacteria, explain why the following statements are true in 1-2 sentences.

Gram positive bacteria are more sensitive to lysozyme and penicillin than gram negative bacteria.

Lysozyme and penicillin break down peptidoglycan and prevents peptidoglycan synthesis, respectively (1 pt). Gram negative bacteria have an outer membrane that protects the peptidoglycan from the lysozyme and penicillin; however, since gram positive bacteria do not have an outer membrane, their peptidoglycan is more accessible to lysozyme and penicillin (4 pt).

Gram positive bacteria are more resistant to drying than gram negative bacteria. Gram positive bacteria have many more layers of peptidoglycan than gram negative bacteria. This highly crosslinked structure helps retain water (5 pt)

4. Isolates of the bacterium *P. aeruginosa* from nature typically have a extensive glycocalyx layer; however, upon extended culturing in the lab, the bacteria tend to loose this layer (i.e. the layer is not observed anymore).

What is the glycocalyx?

The glycocalyx is a network of polysaccharides extending from the cell surface (can also be other materials too) (3 pt).

What is the function of the glycocalyx?

The functions include for protection against phagocytosis, dehydration, viruses, etc; attachment; and nutrient reserve (2 pt).

Based on the function of the glycocalyx, propose a reason for why it is not see in strains that have been grown in the lab for many generations.

In the laboratory, the bacterium is subjected to less conditions/compounds from which it needs protection. Additionally, there is not a need for attachment. Finally, the organism is probably grown in rich medium. Based on these factors, there is not a need to expend energy synthesizing the glycocalyx and so organisms that loose the ability to make this may grow faster and outreproduce those that are still making the glycocalyx (evolution in action)! (5 pt)

- 5. You find a bacterium that is sticking to the surgical tubing and infecting hospital patients. If you were designing a drug against this bacterium, what bacterial component would you try to target to stop the bacterium from causing this problem? *Attachment to surfaces is mediated by pili, glycocalyx, S-layers, and fimbriae so I would target any of these structures. An equally acceptable answer would be just to attempt to stop all protein synthesis in generally (although I was really getting at targeting a certain attachment structure). (5 pt)*
- 6. As an environmental microbiologist, you have isolated a new species of bacteria on a trip to the great salt lakes. <u>Briefly</u> discuss 3 different ways in which you would test if the bacterium is an Archeobacterium or a Eubacterium. I would isolate the plasma membrane and determine its chemical composition. If the membrane lipids were isoprenoids in ether linkages to glycerol instead of fatty acids in ester linkages to glycerol, I would conclude that the bacterium was an Archeobacterium. If the membranes were composed of a monolayer instead of a bilayer, I would conclude that the

bacterium was an Archeobacterium. I would also examine the chemical composition of the cell walls of the bacterium. If the bacterium had pseudopeptidogylcan (NAM is replaced by N-acetylalosaminuronic acid, glycosidic bonds are 1,3 vs 1,4), and L-amino acids are in crosslinks). (10 pt)

List two organelles in eukaryotic cells that are thought to have evolved from prokaryotic cells and <u>briefly</u> describe three lines of evidence that supports this theory? *Two organelles that are thought to have evolved from prokaryotic cells are the mitochondria and the chloroplast (4 pt). The lines of evidence that support this are (6 pt)* (1) *rRNA similar to purple photosynthetic bacteria* (2) *circular DNA encoding some but not all <u>mitochondrial</u> proteins (3) prokaryotic-like ribosomes
(4) antibiotics that inhibit bacterial protein synthesis in bacteria, also inhibit mitochondrial protein synthesis* (5) I also accepted the <u>double</u> membrane and the fact that their activities are similar to the metabolic activities observed in prokaryotes in reaction and in location of enzymes

8. Compare and/or contrast the mechanisms in prokaryotes and eukaryotes that mediate at least 5 out of the 6 activities listed below:

4 pt each (2 for prok and 2 for euk)

cell shape: Prokaryotic cell shape is determined by the cell wall. Eukaryotic cell shape is determined by the cytoskeleton, an intricate intracellular network of interconnected filaments including microfilaments, microtubules, and intermediate filaments. Also some eukaryotes have a simple cell wall and/or a pellicle.

cell movement: Prokaryotes move using flagella that consists of a polymerized flagellin. They can also use gas vacuoles to move up and down in water. Eukaryotes move by using a variety of mechanisms including flagella or cilia (composed of microtubules) or microfilament based movement (ameboid).

intracellular trafficking: Prokaryotes do not have an elaborate mechanism for intracellular trafficking. Intracellular movement is via diffusion. The only defined trafficking is that some proteins destined for membranes, the periplasm, or secretion carry a signal sequence to indicate this. In contrast, eukaryotes use ER, Golgi, and secretory vesicles for sorting and transport of molecule. Microtubules and microfilaments are used for movement of vesicles.

protein synthesis: Protein synthesis occurs on ribosomes in both prokaryotes and eukaryotes. Although the ribosomes are similar in shape, the protein subunits are different and the size of the entire ribosome is different.

In eukaryotes there are several different types of ribosomes. Ribosomes associated with the ER synthesize membrane proteins, proteins to be secreted and some organelle proteins. Ribosomes free in the cytoplasm synthesize cytoplasmic proteins and organelle proteins. Ribosomes in the mitochondria or chloroplasts synthesize organelle proteins.

uptake of nutrients: Prokaryotes have porins other membrane proteins that take up nutrients. Eukaryotes take up nutrients via endosomes.

digestion of large polymers: *Prokaryotes secrete enzymes to digest the polymers and then transport in the monomers; eukaryotes take up the polymers by endocytosis and then the endsome becomes as lysosomes where digetion takes place.*

- 9. You have identified an agent that infects human nerve cells. Provide three ways to <u>help</u> answer the question is the infectious agent a virus or a bacterium?
 - 1. Examine the tissue by electron microscopy to look for viral particles (capsid) or for bacterial cells.
 - 2. Isolate the agent and determine whether it contains onlt one type of nucleic acid (either RNA or DNA) and is therefore a virus. Bacteria have both DNA and RNA.
 - 3. Filtration of agent through filter suggests but does not prove that the agent is viral.
 - 4. Differential centrifugation suggests but does not prove that the agent is viral.
 - 5. Hemagglutination assay strongly suggests virus
 - 6. If the agent can be grown on a plate without host cells it is bacterial.

Note that using bacteria to see if the virus would infect and form plaques was wrong because this is an agent that infects human cells, not bacteria cells.

10 pt

 Temperate phage plaques are frequently cloudy/turbid (as opposed to clear like you saw for T7). Based on what you know about how plaques form, explain why temperate phages may have this plaque type.

Temperate phage can grow either lytically (where they kill the cell) or lysogenically (where they integrate into the chromosome and are now part of the bacterial DNA and the bacteria live). The cloudy plaque results because of some of the phage are going through a lytic lifecycle and lysing cells while others have become lysogenic. The lysogens are now immune to further phage infection and will grow in the plaque causing turbidity (10 pt).

11. You have isolated mutants of bacteriophage lambda that do not form lysogens. There are three possible lambda genes that if nonfunctional (mutated) would give this phenotype (characteristic). List these three genes and explain why lambda that carry nonfunctional (mutated) copies of them can not lysogenize bacteria.

(1.3 pt for gene and 2 pt for explanation) int: If there is no integrase gene, the phage can never form a lysogen because it can not integrate its DNA into the chromosome. cI: If there is no lambda repressor, then the lytic genes expressed from P_R are never shut off and lysis will result. cII: CII is required to turn on the expression of int from P_{int} and of CI from P_{RE} (see above) cIII: CIII stabilizes cII (see above)

I also accepted mutation in N and upregulation mutation in Cro. A mutation in P_{RM} would cause no synthesis of CI (see above). This was only acceptable if you did not also list cI as an answer. 12. Each row in the table below represents a different state of the lambda operator region. Specifically, whether each promoter is turned on or off is indicated, and whether Cro or CI is bound to each operator site is indicated.

For each row below (a-d), predict the growth stage of lambda (i.e. lytic, lysogenic, very early, etc) when the operator sites are filled as shown for that row. Also, provide a <u>brief</u> explanation for your each of your answers.

condition	P _{RM}	OR3	OR2	OR1	PR
a	OFF				ON
b	ON		CI	CI	OFF
с	OFF	Cro			ON
d	OFF	Cro	Cro	Cro	OFF

0.5 pt for stage and 2 pts for explanation

a. very early in infection: The lack of CI or Cro suggests that neither has built up to levels that would allow for binding to the operator sites.

b. lysogeny: The presence of CI at OR1 is preventing expression from P_R and thus is keeping lytic genes turned off. Also, the presence of CI bound to OR2 is stimulating transcription from P_R and thus of cI (repessor of lytic genes through P_R).

c. I accepted either early lytic or predecision. The presence of Cro bound to OR3 <u>suggests</u> that it may "win the race" for occupying the operator.

d. late lysogenic: The presence of Cro atOR3 is preventing expression of CI from P_{RM} and so no repression of lytic growth. The presence of Cro at OR1 is repressing expression of the head and tail genes but that is ok because they have already accumulated to the levels that were needed. In fact, packaging is probably already occurring.

13. Clearly state the difference between plus and minus stranded RNA viruses (using a nucleic acid example if needed).

Plus strand is the same sense as the mRNA (i.e. 5'AUG UUU CCC GTC 3'). Minus strand is the complementary strand to the mRNA (i.e. 3'UAC AAA GGG CAG5'). (5 pt)

What special problems do minus strand RNA viruses face when they enter the cell, and how do they solve the problem?

Minus strand RNA viruses must be transcribed to plus strand RNA to be translated into protein. This is done with a transcriptase (RNA dependent RNA polymerase); since nonplant eukaryotic cells do not contain this enzyme, the virus must carry it into the cell in the capsid (5 pt).

14. You are ready to start a bacterial culture for a very important experiment, but there is no more TSB medium in the laboratory. Joe Shmo, who you know to have BAD aseptic technique, offers you a bottle of TSB medium that he has been using (i.e. removing aliquots). He "claims" that the medium is sterile. What is the best way to verify that he is right (i.e how would you tell if the medium is sterile) before you use it? Incubate an aliquot of the medium overnight and look to see if anything grows (5 pt).

15. As a microbiologist at the Texas Department of Health, you are investigating an outbreak of food poisoning at a local taco joint "Botulism Bell". You take a sample of the picante sauce and make three 1/10 dilutions as follows. You plate 0.1 ml of each dilution tube on a nutrient agar plate and find that tube #3 gives you 100 CFU. Based on the CFU that you counted, how many microorganisms does the picante sauce have in it?

 $(10 \, pt)$

CFU/ml in picante =

<u>CFU counted</u> = 100 = 10^6 CFU/ml = approx 10^6 microbes/ml ml plated * dilution $0.1ml * 10^{-3}$