**Figures/Results Questions**

***Genome Organization and Characterization of Mycobacteriophage Bxb1***

***Mediavilla et al., 2000***

***Halo formation by Bxb1***

1. By examining Figure 1, what did the authors find unique about Bxb1?
2. What type of phage (lytic/temperate) is Bxb1 more likely to be upon examination of Fig. 1?
3. The authors propose two different hypotheses for the observed halo formation. Cite each of these hypotheses.
4. To discern between the two hypotheses, the authors set up a second experiment as outlined in Figure 2A. As part of their experiment, they used a Bxb1 clear plaque mutant. Describe how the clear plaque mutant (Bxb1c1) differs from Bxb1.
5. Briefly outline (words/simple diagram) the concept of the experiment and the outcome for Figure 2A.
6. What was the purpose for obtaining the EM images of Bxb1 (Fig. 2B)?

***Bxb1 is heteroimmune to L5 and D29***

An interesting area of research involves the study of phage immunity (i.e. how one phage prevents another phage from co-infecting M.smeg). While there have been several interesting findings, this is a rather underexplored area of research with regards to mycobacteriophages.

We already know that a phage can contain an integrase gene within its genome that assists with integrating the phage genome into the genome of the *M.smeg*. In order for a phage to be considered a temperate phage, the temperate phage genome must also contain a gene for a repressor protein. The repressor protein binds to numerous binding sites (stoperator sites) along the genome and prevents transcription of genes necessary phage particle production.

Heteroimmunity describes the condition in which a phage that can infect another phage lysogen.

Homoimmunity is defined as the condition in which a phage CANNOT infect another phage lysogen.

Heteroimmunity and homoimmunity are determined by first generating stable lysogens and then testing specific phage to see if they can infect the stable lysogen and generate plaques.

1. In Figure 3, the authors examined susceptibility of wildtype M.smeg, L5 lysogens, and Bxb1 lysogens to infection. Were all phage tested capable of infecting wildtype M.smeg? How do you know?
2. Were all phage capable of infecting L5 lysogens? If not, which phage could infect L5 lysogens?
3. Were all phage capable of infecting Bxb1 lysogens? If not, which phage could infect Bxb1 lysogens?
4. What can be learned from studying the heteroimmunity and homoimmunity of phage?

***Host range specificity***

Another exciting area of research is the host range specificity and receptor usage of phage. There is little known about the specific receptors phage use to enter into their host bacterium. Of particular interest is the identification of the specific receptors mycobacteriophage use to infect the slow-growing mycobacteria (*Mycobacterium tuberculosis*) versus the fast-growing mycobacteria (*Mycobacterium smegmatis*). In this section of the paper, the authors have identified several mutants of wildtype *M.smegmatis*. They noticed that like wildtype *M.smeg*, two of the mutants can be infected with phage D29, while three other mutants were resistant to D29 infection. This suggests that in the three mutant *M.smeg* strains, the authors had managed to knock of a gene or series of genes that code for proteins that the phage uses to bind to and enter the bacterium.

1. Using the data represented in Table 1, what does it mean if one of the *M.smeg* strains is susceptible to infection? Resistant?
2. Why is it not surprising that the patterns of susceptible infection are similar for D29 and L5?
3. What is interesting about Bxb1?

***DNA sequence of the Bxb1 genome***

In this section of the paper, the authors open with a paragraph that describes the sequencing method used to generate the full-length genome sequence of the mycobacteriophage. At the end of the fall term, we will have one of our phage genomes sequenced as well. You will learn more about the sequencing process in the Spring term, so do not get caught up in these details now.

ORF stands for Open Reading Frame. An open reading frame is a section of DNA that codes for amino acids and does not contain any stop (TGA, TAG, TAA) codons. In this case, they are using ORF to indicate putative genes.

1. After reading the section “DNA sequence of the Bxb1 genome” and looking at Figure 4, what do we know about the spacial arrangement of the genes? Is there a lot of “extra DNA” in a mycobacteriophage genome? Alternatively, are the genes tightly packed? Why do you hypothesis phage have this type of genome?

Table 2 is a list of the putative genes identified in Bxb1. NDM stands for No Determined Match. You should notice that many of the genes have no known match to any previous phage and an even greater number of gene functions are unknown. This is what makes phage biology so interesting! There are so many things yet to be discovered!

1. Select one of the known gene functions from Table 2 and look up the function of that protein. Be prepared to report to the class the protein you selected, the function of that protein, and why you think a phage may need to encode a gene that produces that protein. Use the Internet to search your protein here. A smart Google search should be all you need.
2. Take a look at Figure 4. This is a pictorial representation of the Bxb1 genome. Be sure to read the figure legend and try to identify the features being discussed. In the spring, we will use a series of bioinformatics tools to draw a similar map of our class phage.

***Bxb1 structure and assembly genes***

You will not be responsible for this section of the paper or any remaining sections, but you may want to skim them over to familiarize yourself with the style of writing used in a scientific paper. We will be doing quite a bit of scientific writing in the spring.

1. Before putting down this assignment, take a look at Figure 5. Figure 5 is a comparison of the nucleotide sequence of Bxb1 to L5. This is another tool we will use in the spring to examine our class phage in greater detail. The figure is generated by a computer algorithm that compares the first eleven nucleotides of genome 1 (1-11) to the first eleven nucleotides of genome 2 (1-11). If at least nine of the 11 nucleotides match, a dot is generated on the grid. The algorithm then slides down one nucleotide and compares nucleotides 2-12 of genome 1 to nucleotides 1-11 of genome 2. This continues until the entire genome of phage 1 has been compared to the genome of phage 2. Where the phages share a high degree of sequence similarity, you will see a diagonal line in the grid. These plots are relatively easy to generate and we will study them more in the spring.
2. By examining Figure 5, which region of the genomes of Bxb1 and L5 are most similar?
3. Which areas are most different?

**Be sure to come to class prepared to discuss the figures discussed in this guide.**