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From computer to bench work: Is there evidence for a gene product upstream of gene product 1 in Mycobacteriophage Euphoria?

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Introduction:

Mycobacteriophage are a diverse group of viruses that infect mycobacteria. They are the most abundant life-form on earth and are found in many different environments (2). The large collection of mycobacteriophage collected in recent years. In the last century, there has been increased interest in these unique species and the peak in interest has led to an explosion in the research done with phage. Much of this interest is related to the study of *Mycobacterium tuberculosis*. This human pathogen is thought to have infected up to 1/3 of the human population and when left untreated can lead to chronic infection and death (4).

The endless questions about phage are due to the great deal of variety among mycobacteriophage. They vary largely in the genes they express as well as some variation in their physical size. They have been categorized by scientists into clusters based on their genome organization. Phage in the same cluster tend to share unique genes, such as the repressor that will be looked at in this experiment. Despite their genomic similarity, phage within the same cluster do not necessarily share physical similarity in terms of physical structure and the plaques that they produce when they infect mycobacteria.

Phage themselves cannot replicate so they require a host bacteria for the prorogation of new phage. When a mycobacteriophage infects mycobacteria, the process begins by the phage binding to the bacteria and injecting phage DNA into the cell. After the DNA is in the bacteria, the process can be lytic or lysogenic. In the lytic cycle, the bacterial replication mechanism then makes copies of the phage DNA. The phage are assembled within the bacterial cell and finally, they lyse the cell and the phage are released to infect more bacteria (HHMI manual, 2010). In the lysogenic cycle, instead of replicating the phage DNA, it is inserted into the bacteria DNA and the bacterial DNA with phage DNA is replicated, creating a new lysogen (5).

Both mechanisms for replication result in plaques, or clearings, being formed on the bacterial lawn. However, the morphologies for the plaques are very different. The lytic cycle produces completely clear plaques while the lysogenic cycle produces plaques that are cloudy or turbid. Some phage incorporate both mechanisms and these are called temperate phage (5).

Euphoria is a temperate, A cluster mycobacteriophage isolated from a sample of compost pile material from Easton, PA. It was isolated as part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program, sponsored by the Howard Hughes Medical Institute. Euphoria was purified during the fall of 2010. Genomic DNA was isolated and sequenced at the David H. Murdock Research Institute. The genome, consisting of 53,597 base pairs, was annotated by the SEA class at Lehigh University in the spring of 2011 (1).

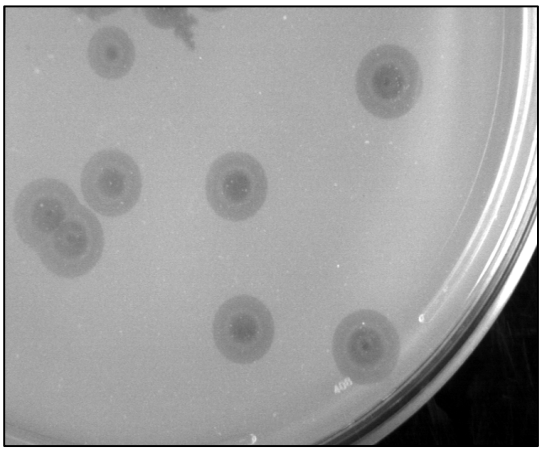

Figure 1 – Euphoria plaques on LB agar

The authors of the Euphoria genome submitted the annotation with the inclusion of an additional gene upstream of what is now called gene 1 (gp1). Our decision to call this open reading frame (ORF) a gene was based on an analysis using bioinformatics tools and the trends determined from the annotation of other bacteriophage genomes. The region is highly conserved within mycobacteriophage Jasper and other A cluster phage (see figure 2). Although this open reading frame is found in several other A cluster phage, our collaborators at the University of Pittsburg did not include this ORF in the final genome submission to GenBank. Their reasoning is that the ORF lacks a strong codon bias typical of the *Mycobacterium smegmatis* host.

Figure 2 - Sequence comparison of Euphoria (top) and Jasper

Hypothesis:

The ORF upstream of the current gene product 1, (bp 26-293), is an expressed gene in *Mycobacteriophage Euphoria*.

Aims:

The goal of this project is to determine if the upstream ORF is expressed using two methods.

1) A reverse transcriptase PCR approach to detect the presence of a corresponding RNA transcript during the Euphoria infection cycle.
2) A recombineering mutagenesis approach to delete the upstream ORF to determine if it is necessary for Euphoria infection of *M. smegmatis*.

Using these two approaches, we aim to gather information about this region of the genome and its potential to encode a product. Our methods will attempt to determine if the gene is transcribed, if it gets translated and when during the infection process the mRNA may be produced.

Experimental Approach:

We will use two different experimental approaches to determine if a gene product is produced. Each method will provide different results to help us answer our many questions.

The first method is reverse transcription polymerase chain reaction (RT-PCR). We will set up a time course experiment where we begin by infecting a culture of *M. smegmatis* with a lysate of Euphoria. Small samples will then be taken at a regular time interval of 10 minutes for three hours. The samples will be pelleted and resuspended in TE before we use a freeze-thaw cycle and bead beating to break the cells open. RNA will be extracted using a phenol-chloroform protocol. We will then perform PCR on the samples in order to determine the presence of different transcripts at different times during the infection. Primers will be created for the cDNA corresponding with the open reading frame of interest as well as for other genes for a control.

We would expect to see some evidence of gene product at the RNA level as evidence that this portion of the genome does encode a product. A prediction as to the function of the gene may also result based on the relative time frame in which it appears. The control genes will provide context for this potential function. If no RNA is present, our hypothesis would not be supported by this experiment. This would suggest that the open reading frame does not encode a product or the product is not detectable using this method.

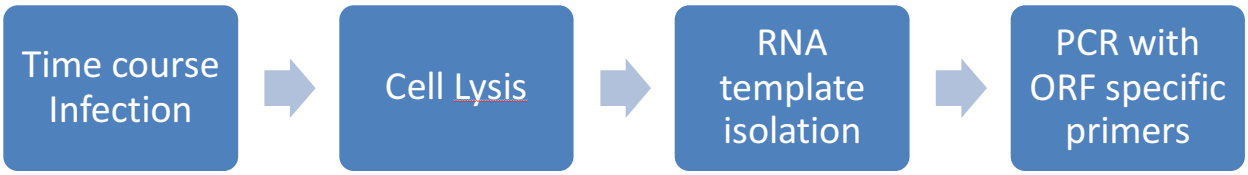

Figure 3 – RT-PCR Approach

The second method we will use is called Bacteriophage Recombineering of Electroporated DNA (BRED). This versatile technique allows us to create mutant versions of the bacteriophage (3). We would use this method to either delete or tag the potential gene. Deleting the gene may affect the cycle of infection or result in a different type of plaque. If this is the result, it would support our hypothesis that a gene product forms. It would also provide us with information about the function of this gene. On the other hand, it may have no effect that we can determine based on the physical characteristics we know. This would not be conclusive, as it would be a limit of the experiment.

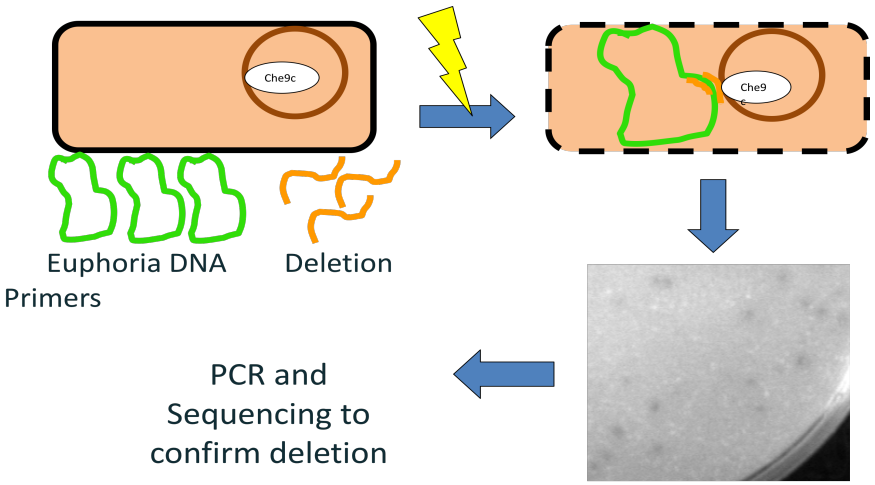

Figure 4 – BRED process

The other option is to tag the open reading frame. We would then attempt to recover the protein with this tag as the gene product. The gene product would not necessarily be a protein, so this method could also result in inconclusive results.

Overall Impact:

The results of this experiment will be important in determining if this ORF encodes a gene product. Results could also affect other A cluster phage with the same open reading frame. A gene product from Euphoria may suggest a gene product in the other similar A cluster phage. This project may contribute to genomic revision for Euphoria and other cluster A phage.

Summary of Results to Date:

This is an ongoing project that will continue in future semesters. Progress has been made, despite the lack of conclusions at this point.

Currently, there is an established protocol for the BRED process that has been shown to work with other phage used at Lehigh University. Despite many trials, there is little evidence that the process is effective at an efficient rate for Euphoria. One trial resulted in plaques. After flooding the plate and plating the lysate there was no evidence that phage were present in the lysate. PCD was done to try and detect any phage particles in the lysate and there was some evidence to suggest that the phage are present in a low titer. We hypothesize that the electroporation process affects Euphoria in a way that prevents future infection. This could include preventing the plasmids from being able to recircularize or infect due to damage to the plasmid. Future work will be done with the lysate and continued efforts will be made to efficiently electroporate with the Euphoria genome.

The RT-PCR process has also been started. Primers have been ordered to detect the upstream ORF of question. A time course infection of Euphoria lysate into *M. smegmatis* has also been done. The samples are stored in a freezer until a nucleic acid isolation can be completed. The primers can then be used to detect the presence of nucleic acid as mentioned in the approach.

Acknowledgements:

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