The Effects of L protein on MreB and Cell Lysis

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Abstract: The MS2 bacteriophage is a single-stranded, positive-strand RNA virus that contains four genes: *mat*, *coat*, *rep*, and *L*. The L protein is responsible for cell lysis, although relatively little is known about its mode of action. Unlike other viral lysis proteins, which inhibit cell wall synthesis at the division site causing midcell blebs, L protein appears to cause lysis at random cellular locations, as seen by bleb formation throughout the cell. We hypothesized that L protein works with MreB, an essential protein for cell wall synthesis, which is localized throughout the cell body. We seek to identify how L protein affects MreB by possibly: causing the mislocalization of MreB at specific sites of lysis, activating a section of MreB to form a hotspot for cell wall synthesis, or deactivating a section of the MreB pool, causing cell defects. To begin to determine which mechanism is correct, we will measure the localization of MreB in cells undergoing lysis to see if there is a correlation between MreB localization and L protein induced lysis. We will then examine if there is a direct interaction between L protein and MreB using biomolecular fluorescent complementation. Learning how L protein lyses cells will provide us with a better understanding of single gene lysis, which can be applied to phage therapy to kill disease causing bacteria and to effectively prevent bacteriophages from killing helpful bacteria that assist in preventing disease or are important for industrial purposes.

Keywords: L Protein, Mreb, Single-Gene Lysis System, Mislocalization, MS2 Phage

Introduction

Bacteriophages are viruses that contain either a DNA or RNA genome that infect and replicate within bacteria, moving between cells while surrounded by a protein capsid. The virus can overcome the infected cells defenses to begin the transcription and replication of the viral genome. The bacteriophage that we are focusing on, MS2, is a single-strand RNA phage that specifically infects conjugation competent cells by binding to the F pilus (Usui et al. 2015). The MS2 phage is one of the most studied phages and was sequenced completely in 1979 (Fiers et al. 1976). It is 3,569 nt long and contains four main genes: *mat*, *coat*, *rep*, and *L*. Because the phage is a positive sense phage and uses single-stranded RNA as its genetic material, it's genome acts as messenger RNA and can immediately begin translating viral protein within the host cells (Poot et al. 1997). However, in order to replicate the genome, replicase is needed to produce the complementary minus strand RNA, which can be used as a template to make the new positive strand (Peabody 1993). The coat protein forms the majority of the viral capsid. It also acts a translation repressor late in infection by binding to a specific stem-loop structure in viral RNA containing the ribosome-binding site (Witherall et al. 2000). The capsid also includes one copy of the maturation protein (*mat*), which is needed for binding to the bacterial pilus (Dent et al. 2013).

In order to escape the cell once viral replication is complete the virus must lyse the cell using L protein. While the roles of the genes *mat*, *rep*, and *coat* are very well known, not much is known about the function of L protein. The L protein is a single-gene lysis protein that kills cells by making holes in the membrane and cell wall (Bertani 1950). As a single-gene lysis protein L must first form a pore in the inner membrane and then somehow induce the process of lysis, eventually killing the host bacterium

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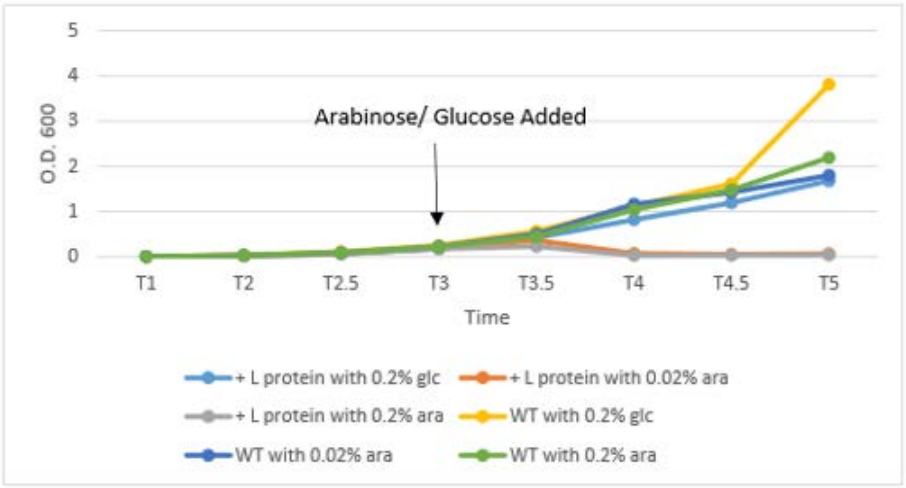

Figure 1 – The cells were grown for 3 hours and either arabinose or glucose was added. The cells began to die between T3.5 and T4 and only cells treated with arabinose. The time is shown in hours.

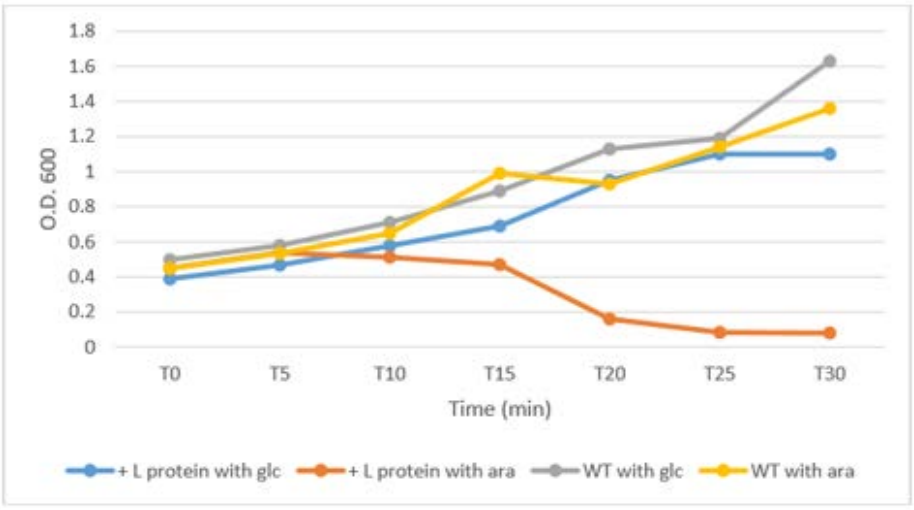

Figure 2 – The cells were grown for 3 hourse (not shown). Then either glucose or arabinose was added at T0 and measurements were taken every 5 minutes to more accurately determine the rate of death.

(Horkami et al. 1997). The L protein is 75 amino acids long but only the first 25 amino acids correspond with the process of lysing and killing the cell (Jou 1972). The model for L-mediated lysis begins with the L protein forming pores within the inner membrane and then acting as an inhibitor for the synthesis of the peptidoglycan wall that then induces the process of lysis, destroying the cell (Pazmany et al. 1996).

While other single-gene lysis systems target proteins involved with the formation of the division septum, leading to localized lysis at the division site, L protein does not. Instead, the membrane blebs that are produced by the induction of lysis are seen throughout the cell body. We therefore hypothesize that L protein works through MreB, an essential protein for cell wall synthesis that is localized throughout the cell body. When MreB is removed or deactivated within a bacterium, the cell loses its rod shape and becomes rounded. However, cells that have undergone lysis through the L protein generally do not lose rod-shape, which suggests that L is not simply deactivating all of the cellular MreB. Possible models for how L protein could affect MreB are: a) L protein causes the mislocalization of MreB at specific sites in the cell, b) L protein activates a subsection of the total cellular MreB pool forming a hotspot for cell wall synthesis leading to lysis or c) L protein deactivates a subsection of the total cellular MreB pool causing localized cell defects which lead to lysis.

Methods

Strain Construction

An *E. coli* strain expressing MreB-GFP (chloramphenicol resistant) was transformed with a plasmid (ampicillin) containing L protein under an

arabinose inducible promoter. Cells were selected on ampicillin (100µg/ml) and chloramphenicol (30µg/ml) media so that only the cells with the L protein plasmid would grow.

E.coli are not susceptible to vancomycin because the outer-membrane blocks entry of the drug, however, mutations that disrupt the outer-membrane allow entry of vancomycin (Matsuyama 1997). We transduced MreB-GFP into a strain of *E.coli* containing a mutation in *lptD*.

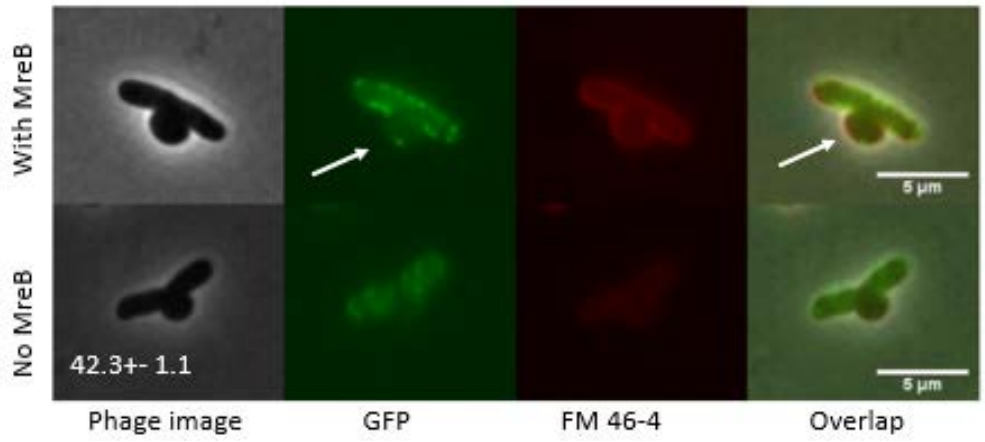

Figure 3 – A strain of E. coli *was grown for 3 hours and then the drug vancomycin was added for 20 minutes to induce bleb formation. The blebswere then assayed for the presence of MreB. Image taken by Alannah Templon.*

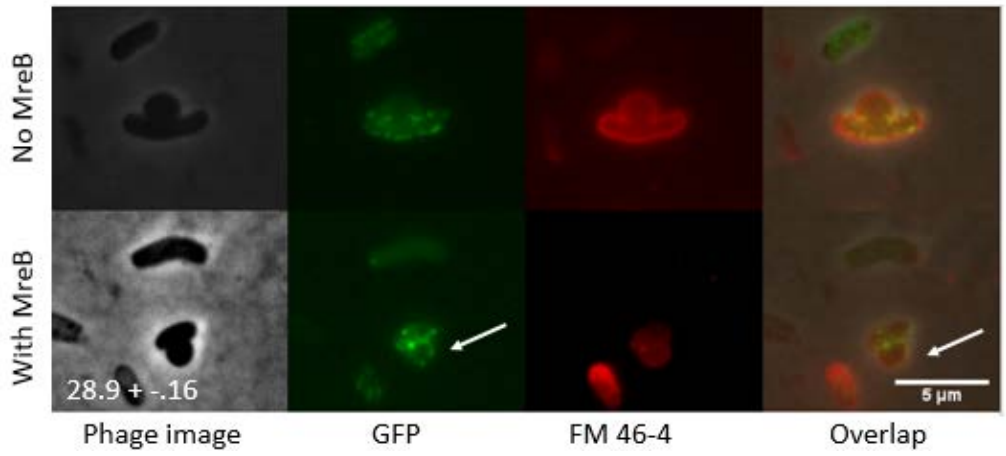

Figure 4 – Cells containing L protein were grown for 3 hours and then arabinose was added to induce L protein production. Cells were grown in arabinose for 35-45 minutes and then blebs were assayed for the presence of MreB. The arrows indicate the presence of MreB in a bleb. Image taken by Alannah Templon.

Growth Curve

E.coli cultures were grown overnight in a 37^{O}C shaking incubator. WT cells (with an MreB-GFP fusion) were grown in LB broth supplemented with chloramphenicol (30µg/ml), while a strain with an L protein transformed plasmid was grown in LB supplemented with ampicillin (100µg/ml) and chloramphenicol. Each strain was diluted 1:1000 in a beaker of LB media. Strains were grown for 3 hours and then either 0.2% or 0.02% of glucose or arabinose was added to each beaker. The experiment was done in triplicate (Figure 1, Figure 2).

Green Fluorescent Protein Microscopy

Both GFP and FM4-64 dye were used to visualize the MreB and the cell membrane of the *E. coli* cells, respectively. FM4-64 was added at a final concentration of 5 µg/ml. (Figure 3) *lptD* mutant cells were grown overnight and subcultured for 3 hours in LB media before vancomycin was added in a 20 µg/ml concentration. 40 minutes after vancomycin was added, images were taken under the microscope and blebs with and without MreB were counted. (Figure 4) Cells expressing L protein were grown overnight and subcultured 1:1000 for 3 hours. Arabinose

was added to induce L protein for 40 minutes. Images were taken under the microscope and blebs with and without MreB were counted.

Results

Induction of L Protein Causes Cell Death Quickly

We performed growth curves with both WT *E. coli* cells and cells with the L protein transformed plasmid. Time points were taken every hour for 2 hours and then every 30 minutes for 3 hours. The results of the growth curves show that after arabinose was added, induction of L protein leads to a decrease in the growth rate of cells after 30 minutes (Figure 1). One hour after the addition of arabinose, the majority of cells had lysed. To better track cell death, the same experiment was performed with time points taken in 5 minute increments after the addition of arabinose. We observed that 10 minutes after the addition of arabinose cell growth began to slow and after approximately 20 minutes the cells were mostly dead (Figure 2). As a control, glucose was added to the cultures. L protein strains with glucose continued to grow because the glucose does not induce the expression of L protein. WT strains continued to grow regardless of the addition of arabinose or glucose because it does not have the L protein, and the addition of either sugar is not lethal.

L Protein Produces Blebs with MreB Less Often Than the Control

To test the model that L protein causes the mislocalization of MreB at specific sites, *E. coli* were imaged after expression of L protein for 40 minutes. There are three possible outcomes from this experiment. First, L protein is recruiting MreB, which would result in 100% of blebs containing MreB. Second, L protein is repelling MreB from certain sites, which would result in 0% of blebs having MreB. The third model is that L protein has no effect on MreB localization, which might result in a mixed percentile of blebs with MreB. We observed that about 38% of the blebs contained MreB (Figure 4), suggesting that L protein was expelling MreB from certain site in the cell.

Vancomycin Induced Blebs Contain MreB Appx. 45% of the Time

In order to test that the expulsion of MreB from blebs was due to L protein and not a function of bleb formation, we treated cells with the cell wall inhibitor, vancomycin. Images of *E. coli* cells treated with vancomycin were taken under the microscope and showed that about 45% of the blebs contained MreB (Figure 3). This is significantly higher than the experimental group ($P < 0.05$) (Figure 4), suggesting that L protein is causing the removal of MreB from the sites of blebs formation.

Discussion

Through these experiments we have been able to make several conclusions while continuing to further our research. We were able to conclude that L protein causes cell death approximately 20 minutes after induction. Additionally, vancomycin induced blebs contain the actin homologue MreB approximately 45% of the time. Lastly, L protein induced blebs contain MreB less often than those induced by vancomycin, occurring only 38% of the time. This suggests that the model of L protein mislocalizing MreB may be occurring in the cells.

However, our research strives to show whether or not there is a definitive correlation between MreB localization and L protein. If L directly activates or represses MreB activity than they most likely interact with each other. We will use a bimolecular fluorescence complementation (BiFC) assay to determine if L protein and MreB interact. A positive localized interaction would support the hypotheses that L protein regulates a subsection of MreB. Second, we will determine if MreB localization changes during lysis.

This research is necessary because little is known about the L protein. Through this research we will see if there is an interaction between MreB and L protein leading to a better understanding of single-gene cell lysis. A practical application includes phage therapy, which can be used to kill disease-causing

bacteria. Additionally, bacteriophages are harmful in industries that rely on bacterial growth, i.e. yogurt making. Through acquiring a better understanding of bacteriophages, we can more effectively attack and kill certain diseases and infections and prevent bacteriophages from killing helpful bacteria.

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