A SHIFT IN THE LYSIS PARADIGM:

HOW PHAGE ΦKT BREAKS FREE

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

A Shift in the Lysis Paradigm: How Phage ΦKT Breaks Free

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Spanins are required for the last step in bacteriophage lysis: the disruption of the outer membrane. However, bioinformatic analysis has shown 15 percent of phages lack a spanin gene, which suggests an alternate mechanism of outer membrane disruption. To address this, we selected virulent podophage ΦKT as a spanin-less exemplar and tested ΦKT genes for outer membrane disruption during lysis. Hypothetical novel gene *28* causes outer membrane disruption when co-expressed with ΦKT lysis genes and complements the lysis defect of a λ spanin mutant. Gp*28* is a 56 aa cationic peptide with predicted amphipathic helical structure and is associated with the particulate fraction after lysis. Urea and KCl washes did not release gp*28* from the particulate, suggesting a strong hydrophobic interaction with the membrane. Furthermore, membrane interaction was demonstrated using functional gp*28*-sfGFP, which localized to the periphery of the cell. Based on this data, gp*28* represents a new class of lysis proteins, a disruptin – an antimicrobial peptide produced to destroy the outer membrane during lysis. This is the first reported phage-encoded antimicrobial peptide.

ACKNOWLEDGEMENTS

I would like to thank Dr. Young for his mentorship and the opportunity to work in his lab as an undergraduate researcher. I would also like to thank Jesse Cahill for his contributions and guidance throughout the course of this project. Thank you also to all of my family and friends for their encouragement and support on my journey to the completion of this thesis.

CHAPTER I

INTRODUCTION

Bacteriophages, or phages, are the viruses of bacteria. Like all viruses, phages propagate through three steps: infection, replication, and release. Tailed phages are released from bacterial hosts by lysis, the rapid destruction of the bacterial cell (1). For Gram-negative hosts, lysis requires three classes of proteins that target the three layers of the cell envelope (2). Phage λ is a well-studied lysis system and has served as the basis for the current model of lysis (3-5). In λ, the lysis genes are encoded in the late transcriptional unit of the phage (3). This lysis cassette includes four genes, *S*, *R*, *Rz*, and *Rz1*, and these genes produce the three classes of lysis proteins: holin, endolysin, and spanin (4). The first step of λ lysis is the formation of micron-scale holes in the inner membrane (IM) by the holin, encoded by the *S* gene (4, 5). The holin holes allow for the release of endolysin into the periplasm, and endolysin degrades the peptidoglycan layer (PG) (3). Following degradation of the PG, the spanin disrupts the outer membrane and completes the final step of lysis (6). The λ spanin complex is composed of two subunits, encoded by *Rz* and *Rz1* (7). Recent experiments have shown the spanins are fusogenic proteins, leading to the model that spanins cause outer membrane disruption by fusing the IM and OM after PG degradation (8, 9).

Both subunits of the spanin are essential for λ lysis (6, 7). At the cellular level, λ lysis can be visualized as an explosive release of the cell contents (6). However, with nonsense mutations in the spanin genes, the cell converts from rod shape to a persistent round shape without releasing the contents of the cell. This indicates that lysis is blocked at the outer membrane disruption step, after degradation of the PG (2, 6).

Spanin gene equivalents have been identified in the genomes of numerous Gram-negative phages, further supporting the importance of spanins in the lysis process (10). Spanins are encoded by either one or two genes, with various genetic architectures (10). Although individual spanin genes display a wide range of diversity, spanins must "span" the cell envelope. Therefore, spanins share common characteristics, including the requirement for a transmembrane domain to localize to the cytoplasmic (inner) membrane and a lipoylated Cys residue for localization to the OM. These features are the best markers available for annotating spanin genes.

Bioinformatic analysis of 677 phage genomes showed 91 of the double-stranded DNA phages do not have annotated spanin genes (11). To address how phages lyse without spanins, we selected phages of *E. coli* with simple genomes, which were small enough to guarantee no cryptic, unannotated spanin gene was present. One of these, phage ΦKT, is a virulent podophage (12). ΦKT has a small genome (~42 kilobases) and infects 4s, an environmental strain of *E. coli*. In this study, we sought to identify the missing lysis gene responsible for OM disruption.

CHAPTER II

METHODS

Strains, phages, plasmids, and primers

The strains, phages, and plasmids used in this study are presented in **Table 1**. The primers used for this study are summarized in **Table 2**.

Table 1. *Strains, phages, and plasmids*.

Name	Genotype and relevant features	Reference or source
Bacteriophages		
ΦKT	Virulent podophage of *E. coli* 4s	(12, 13)
λ900	λΔ(*stf tfa*)::*cat c*I857 *bor*::*kan*, carries Cam^r and Kan^r	(7)
λ900 $Rz_{am}Rz1_{am}$	λΔ(*stf tfa*)::*cat c*I857 Rz_{Q100am} $Rz1_{W38am}$ *bor*::*kan*	(7)
Strains		
4s	Isolated from horse feces	(12, 13)
RY16390	MG1655 Δ*tonA lacIq* Δ*lacY*	Laboratory stock
MG1655(λ900)	RY16390 lysogenized with λ900	This study
MG1655(λ900 $Rz_{am}Rz1_{am}$)	RY16390 lysogenized with λ900 $Rz_{am}Rz1_{am}$	This study
Plasmids		
pRE	Medium copy plasmid containing Q-dependent, λ late promoter pR’	(14)
pQ	λ Q cloned under $P_{lac/ara-1}$ promoter in a low copy number plasmid pZS-24*	(15)
pRE seg 8	pRE containing ΦKT lysis cassette (seg 8)	This study

Table 1. cont.

Name	Genotype and relevant features	Reference or source
Plasmids Continued		
pRE seg 8 gp*28*$_{E17X}$	pRE containing ΦKT lysis cassette with a nonsense mutation at residue 17 of gene *28*	This study
pRE seg 8 Δgp*28*	pRE containing ΦKT lysis cassette with an in-frame deletion of residues 20-42 of gene *28*	This study
pRE gp*28*	pRE containing ΦKT gene *28*	This study
pRE gp*28*-His	pRE containing ΦKT gene *28* with a polyhistidine tag of amino acid sequence GGHHHHHHGG fused to the C-terminus	This study
pRE gp*28*-His$_{E17X}$	pRE containing ΦKT *28-His* with a nonsense mutation at residue 17	This study
pRE RzRz1	pRE containing λ *RzRz1*	(16)
pRE Rz1-His	pRE containing λ *Rz1* with His-tag at the C-terminal end	(16)
pRE gp*28*-sfGFP	pRE containing ΦKT *gene 28* fused to sfGFP	This study

Table 2. *Primers and DNA constructs*.

Primers	Sequence (5'– 3')
For ΦKT seg 8	AGTTCTGGTACCATGCTGATGCTGTGTCACCAT
Rev ΦKT seg 8	GTCTACTGGATCCAGGCATCTAATCAGGGTCAGGT
For gp*28*$_{E17X}$	CACCAAGTAAACATGGTGTGACTATCCTGC
Rev gp*28*$_{E17X}$	CATGTTACTTGGTGAAATCCCATGCG
For gp*28* deletion	CGCCGATGTACTTGATACGGT
Rev gp*28* deletion	CCATGTTCCTTGGTGAAATCCCAT
For gp*28*	AGTTCTGGTACCGGCTGAATGCTCCGTATACACAAC
Rev gp*28*	GTCTACTGGATCCGTGGTCACAAGGGAACCGTAGTTC

Table 2. cont.

Synthetic DNA	Sequence (5'– 3')
gp*28*-His	TAGAGTATCTGGTACCATACACAACTGGAGACTTTAATGAGTAAATTCA AGAAATATCTGGGTGCCGCATGGGATTTCACCAAGGAACATGGTGTGAC TATCCTGCGTGGTGTGGCTGTCCTCCTTGTAGGTCGTAAGGTTGGTCGC GTTGCCAACCAGTCCGCCGATGTACTTGATACGGTCATCAAAGGGACTA AGAACAATGGTGGGCACCACCATCACCATCACGGTGGCTAAAGGTGAAG CTTTAACTAGGATCCTGTTACCACTT
gp*28*-sfGFP	TAGAGTAGGTACCTCTGAATTCATACACAACCGGAGTAGAAGATGAGTA AATTCAAGAAATATCTGGGTGCCGCATGGGATTTCACCAAGGAACATGG TGTGACTATCCTGCGTGGTGTGGCTGTCCTCCTTGTAGGTCGTAAGGTT GGTCGCGTTGCCAACCAGTCCGCCGATGTACTTGATACGGTCATCAAAG GGACTAAGAAGAATAGTGGATCCAAAGGTGAAGAACTGTTCACCGGTGT TGTTCCGATCCTGGTTGAACTGGATGGTGATGTTAACGGCCACAAATTC TCTGTTCGTGGTGAAGGTGAAGGTGATGCAACCAACGGTAAACTGACCC TGAAATTCATCTGCACTACCGGTAAACTGCCGGTTCCATGGCCGACTCT GGTGACTACCCTGACCTATGGTGTTCAGTGTTTTTCTCGTTACCCGGAT CACATGAAGCAGCATGATTTCTTCAAATCTGCAATGCCGGAAGGTTATG TACAGGAGCGCACCATTTCTTTCAAAGACGATGGCACCTACAAAACCCG TGCAGAGGTTAAATTTGAAGGTGATACTCTGGTAAACCGTATTGAACTG AAAGGCATTGATTTCAAAGAGGACGGCAACATCCTGGGCCACAAACTGG AATATAACTTCAACTCCCATAACGTTTACATCACCGCAGACAAACAGAA GAACGGTATCAAAGCTAACTTCAAAATTCGCCATAACGTTGAAGACGGT AGCGTACAGCTGGCGGACCACTACCAGCAGAACACTCCGATCGGTGATG GTCCGGTTCTGCTGCCGGATAACCACTACCTGTCCACCCAGTCTGTTCT GTCCAAAGACCCGAACGAAAAGCGCGACCACATGGTGCTGCTGGAGTTC GTTACTGCAGCAGGCATCACGCACGGCATGGATGAGCTCTACAAATAAA GGATAGGATCCTGTTACCACTTGTGAAGCTTTAAT

DNA manipulation

The pRE seg 8 plasmid was constructed by PCR amplifying the ΦKT lysis cassette with the For ΦKT seg 8 and Rev ΦKT seg 8 primers. These primers contain KpnI and BamHI overhangs and were designed to amplify the section of the ΦKT genome from nucleotide 36,581 to 37,741 (GenBank, NC_ 019520.1). The gel-purified PCR product was double digested with the corresponding restriction enzymes and ligated into pRE using T4 DNA ligase. The complete plasmid was transformed into XL1Blue cells. The pRE gp*28* plasmid was constructed by a similar method, the only modification being that primers For gp*28* and Rev gp*28* were used to amplify from nucleotide 37,067 to 37,323 in the ΦKT genome. Quick change modifications were performed as described previously (17).

Bacterial growth, induction, and lysis monitoring

Bacterial cultures were grown, induced, and monitored for lysis as described previously (17).

ΦKT infection

Bacterial cultures were grown at 37°C to an A_{550} of ~0.3. Then the cultures were put on ice, and phage were added to the specified samples at a multiplicity of infection (MOI) of 30. After 90 minutes on ice, the cultures were again incubated at 37°C and monitored for lysis. **SDS-PAGE and western blotting**

Samples were collected and prepared for Western blotting as described previously (8). After preparation, the samples were resolved on a Novex 10-20% Tricine SDS-PAGE gel (Thermo Fisher). Gel transfer and immunodetection were done using the iBlot and iBind systems (Thermo Fisher) using the manufacturer recommended protocol. For the primary antibody step, 1 μL of mouse anti-His antibody (Thermo Fisher, MA1-135) was added to 2 mL of 1x iBind

solution. A goat anti-mouse (Thermo Fisher, 32430) antibody was used as a secondary antibody, and 2 μL of this antibody was used in 2 mL of 1x iBind solution. Chemiluminescence was detected on an Amersham Imager 600 RGB (GE).

Subcellular localization

Cells were induced at 0.25 A_{550} and 10 mL samples were collected 55 minutes after induction, after the culture had cleared. Cells were placed on ice, and 1 μL of protease inhibitor cocktail (Sigma-Aldrich, P8465) was added to each 10 mL sample. This lysate was spun down at 6,000 x *g* for 5 minutes at 4°C to clear unlysed cells. Then, three 3 mL aliquots of supernatant were taken from each 10 mL sample and spun at 100,000 x *g* for 60 minutes at 4°C in a TLA 100.3 rotor using an Optima MAX-XP ultracentrifuge. After the centrifugation, the supernatant was removed, and 500 μL of 1x PBS, pH 7.4 was added to the pellet. The pellet was left to sit overnight in the PBS at 4°C and, the next day, was resuspended. An additional 2.5 mL of PBS was added to each sample. A second spin was performed at 100,000 x *g* as described above. Again the supernatant was discarded after centrifugation. Next, 500 μL of 2 M KCl, 1x PBS, or 2 M urea was added to the corresponding samples and left to sit overnight. The next day, another 2.5 mL of the same solution was added to each sample. A final centrifugation was performed as described above. After centrifugation, 1 mL of the supernatant was collected and a 10% TCA precipitate was performed as previously described (5, 18). The TCA precipitate pellet was resuspended in 1x sample loading buffer (SLB) at 100 μL per 1 OD unit as measured at 40 minutes after induction of the original bulk culture. The rest of the supernatant from the centrifugation was discarded, and the pellet was normalized to the OD units as described above. Both sets of resuspended pellets were boiled for 5 minutes and run on a gel as described above.

Spheroplasting

Spheroplasts were made as described previously (9).

Phase contrast and fluorescence microscopy

For the spheroplasts, samples were prepared for imaging after completion of the spheroplasting protocol. A 1.5 μL sample was applied to a glass slide and covered with a coverslip. The sample was imaged immediately using a plan-apochromat 20x/0.8 Ph2 objective installed on a Zeiss Axio Observer 7 inverted microscope. For the cells containing pRE gp*28*-sfGFP, sample was collected from bulk culture 40 minutes after induction. Again, 1.5 μL was applied to a slide and covered with a coverslip. Samples were imaged using a plan-apochromat 100x/1.46 objective on a Zeiss Axio Observer 7 inverted microscope. GFP images were captured using filter cube 90 HE and 100ms exposure time at 30% intensity. All image processing was done using the Zen 2.3 software.

CHAPTER III

RESULTS

ΦKT causes rapid lysis in bulk culture

During lysis by phages lacking spanins it has been shown that the outer membrane can be artifactually disrupted by the shearing forces attendant to aerated growth, unless divalent cations in millimolar concentrations are added to the growth media. Therefore, we hypothesized that if ΦKT encodes an outer membrane disruptor, the rate of lysis in magnesium-supplemented media would be the same as standard media.

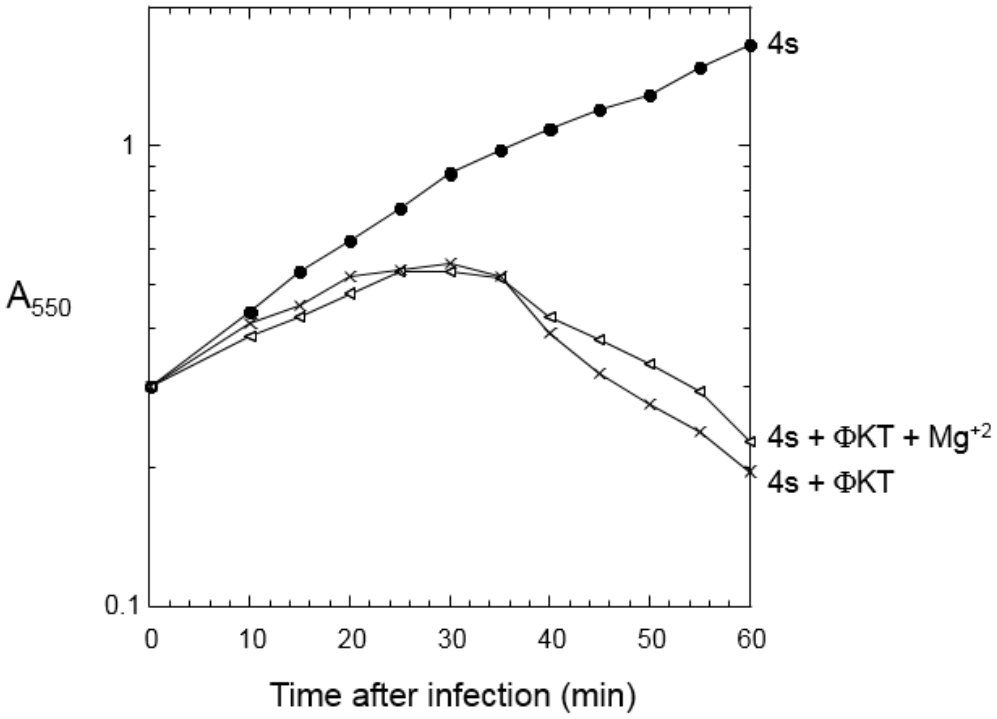

Figure 1. *Infection of 4s with ΦKT.* After 90 min adsorption on ice, the absorbance of each culture was monitored. The cultures included: 4s (●), 4s infected by ΦKT in the presence (◁) and absence (X) of magnesium ions.

Therefore, we infected 4s with ΦKT and monitored the absorbance of the culture for 60 minutes after infection (**Figure 1**). Surprisingly, there was sudden decrease in the culture turbidity at about 30 minutes, which indicated rapid lysis of the cells infected with ΦKT in the presence and absence of magnesium supplementation. Therefore, ΦKT lyses its host under conditions that require outer membrane disruption. This suggests ΦKT produces an outer membrane-disrupting protein.

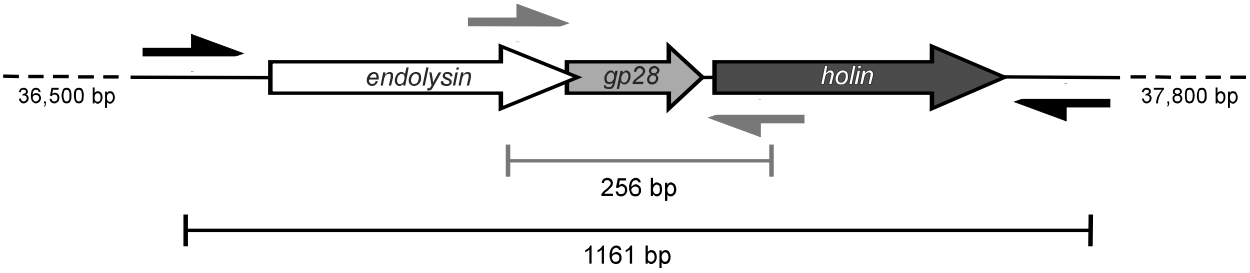

Figure 2. *The lysis region of ΦKT*. The region of the ΦKT genome encoding the putative endolysin and holin, genes *27* and *29* respectively. A hypothetical novel gene *28*, encoding a 56 amino acid product, exists between the other two lysis genes. The 1161 base pair (bp) region was cloned to test the lysis region of ΦKT, and the 256 bp segment was used to test gene *28*.

The endolysin and holin genes of ΦKT have been annotated but not tested. In λ it has been shown that the holin and endolysin are not sufficient to complete the lytic process (6). Therefore our next step was to clone the 1kb gene region containing gene *27* (endolysin), gene *28* (hypothetical novel protein) and gene *29* (holin) (**Figure 2**) and determine whether this apparent lysis cassette could support lysis. Surprisingly, by expressing the ΦKT lysis cassette from a pRE plasmid known to support phage transcription at physiologically relevant levels, rapid lysis of the culture was observed (**Figure 3**). This suggested that one of the genes within

this region acts as an outer membrane disruptor. To address this, we chose to make mutations in gene *28*.

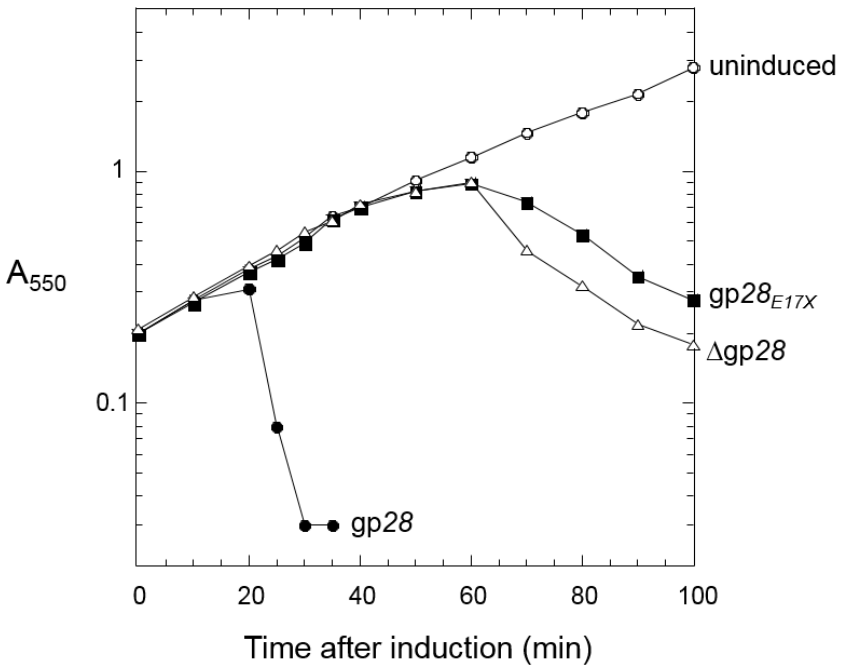

Figure 3. *Induction of lysis cassette in MG1655.* The MG1655 cells, containing both pQ and the respective pRE plasmids, were monitored for absorbance at 550 nm for 100 min. The uninduced, negative control, is shown with open circles (○). The other three cultures were induced with 1 mM IPTG at time 0. The ΦKT lysis cassette construct (●), the lysis cassette with a nonsense mutation at residue 17 of gp*28* (■), and the cassette with an in-frame deletion of gp*28* (△).

Mutations in gene *28* prevent lysis

Two knock-out alleles of gene *28* were constructed by site-directed mutagenesis: a nonsense mutation at residue 17 (28_{E17X}), and a deletion of residues 20-42. As expected, both the nonsense and in-frame deletion mutations of gene *28* blocked lysis, showing a spanin-null phenotype (**Figure 3**) (6). This result strongly suggests that gene *28* is required for lysis.

Gene *28* complements the λ spanin lysis defect

To test the dependence on the other ΦKT lysis genes, gene *28* was cloned into the pRE plasmid, in which transcription is under control of the λ late antiterminator Q. This plasmid was transformed into a MG1655(λ900 $Rz_{am}Rz1_{am}$) lysogen, thermally induced and monitored for lysis (**Figure 4**). Similar to pRE RzRz1, pRE gp*28* restored the lysis phenotype of the spanin mutant, indicating the outer membrane disrupting function of gene *28* is not specific for the other ΦKT lysis genes. This is consistent with the observation that phage lysis proteins are mechanistically independent of each other (19).

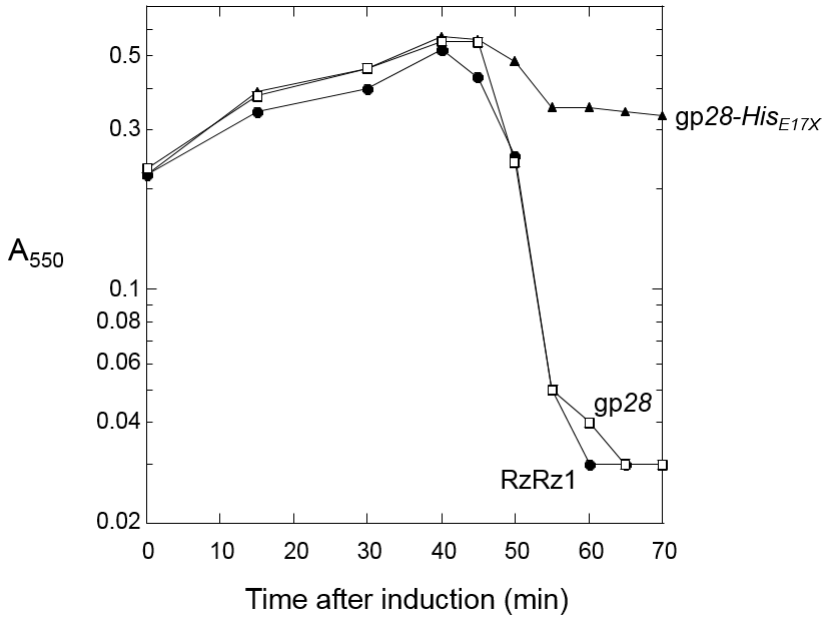

Figure 4. Gp*28 complements λ spanin defect*. MG1655(λ900 $Rz_{am}Rz1_{am}$) carrying pRE gp*28*-His_{E17X} (▲), pRE gp*28* (□), or pRE RzRz1 (●) were thermally induced and monitored for lysis (A_{550}). pRE gp*28*-His_{E17X} acted as a negative control, and pRE RzRz1, as a positive control.

Gp*28* localized to particulate fraction

A polyhistidine epitope tag was fused to the C-terminus of gp*28* to allow for anti-His antibody detection of the protein. The tag has an amino acid sequence of GGHHHHHHGG. The detection of gp*28*-His was verified by Western blot (**Figure 5**).

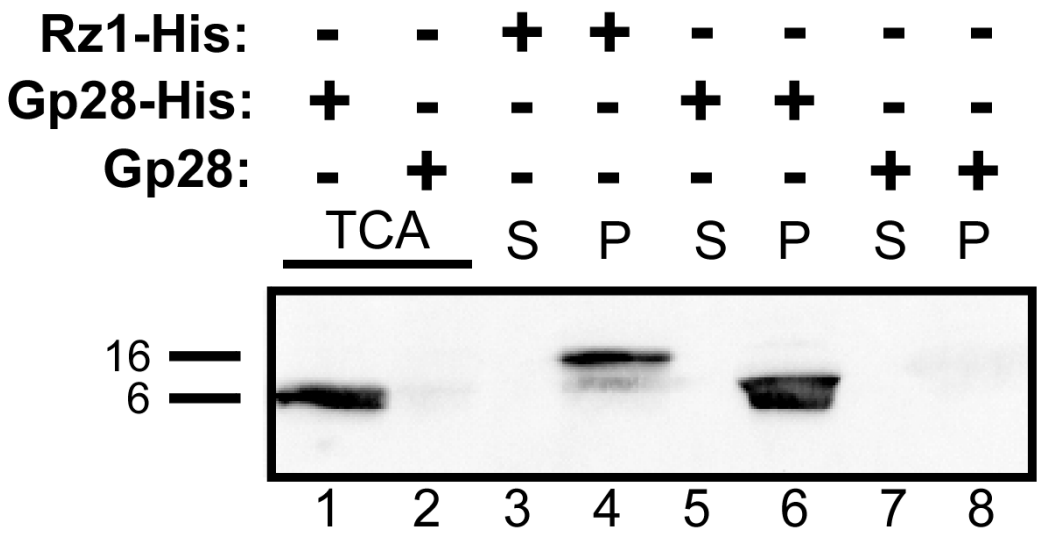

Figure 5. *Localization of* gp*28 to the particulate fraction*. Lanes 1 and 2 illustrate proper immunodetection of the gp*28*-His construct. Rz1-His is present in the particulate fraction (Lane 4) of the lysate rather than the soluble fraction (Lane 3). Gp*28*-His shows a similar trend; gp*28* was detected in the particulate fraction (Lane 6) rather than the soluble fraction (Lane 5). As expected, there was no detection of gp*28* (Lanes 7 and 8).

Based on the previous data, we hypothesized that gp*28* acts directly on membranes. To test this localization of gp*28*, we collected sample after lysis from a culture of MG1655(λ900 $Rz_{am}Rz1_{am}$) containing pRE gp*28*-His. The lysate was subjected to a centrifugation (100,000 x *g* for 60 minutes) to separate the membrane from the soluble material. The soluble and particulate fractions were imaged on a Western blot (**Figure 5**). Gp*28*-His associated with the particulate

fraction. As a control, gp*28*-His was compared with Rz1-His. Rz1-His also associated with the particulate fraction and is known to bind to the outer membrane (7). The presence of gp*28* in the particulate fraction supports the hypothesis that gp*28* is associating with the bacterial membrane.

Gp*28* prevents spheroplasting

To investigate the specific localization of gp*28* within the cell envelope, we attempted subcellular fractionation using a sucrose cushion (20). However, at the spheroplasting step, cells expressing gp*28* lysed (**Figure 6**), whereas control cells did not. By isolating steps of the spheroplasting process, we found that lysis was caused by the exogenous addition of lysozyme. This result is consistent with the prediction that gp*28* interacts with both the inner and outer membranes and suggests that gp*28* is positioned in the membrane prior to holin triggering.

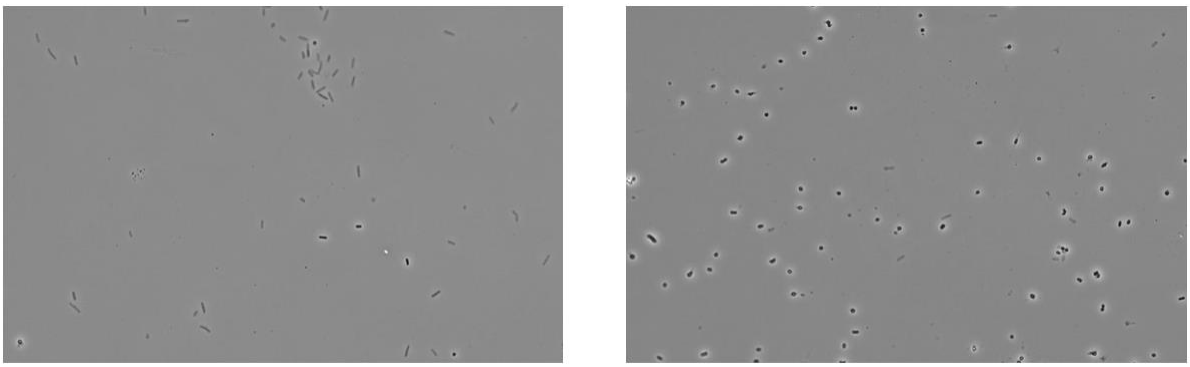

Figure 6. Gp*28 prevents spheroplasting*. Comparison of MG1655 cells containing pRE gp*28* (left) and pRE (right) after spheroplasting.

Salt washes determine nature of interaction

To test the nature of the interaction between gp*28* and the membrane, lysate was collected from MG1655(λ900 $Rz_{am}Rz1_{am}$) expressing gp*28*-His. The particulate and soluble

fractions were separated by centrifugation at 100,000 x *g* for 60 minutes and visualized by Western blot (**Figure 7**). A small amount of gp*28*-His appeared in the soluble fraction of the urea wash, but gp*28*-His remained solely in the particulate fraction for both the PBS and KCl washes. This suggests a hydrophobic interaction with the membrane.

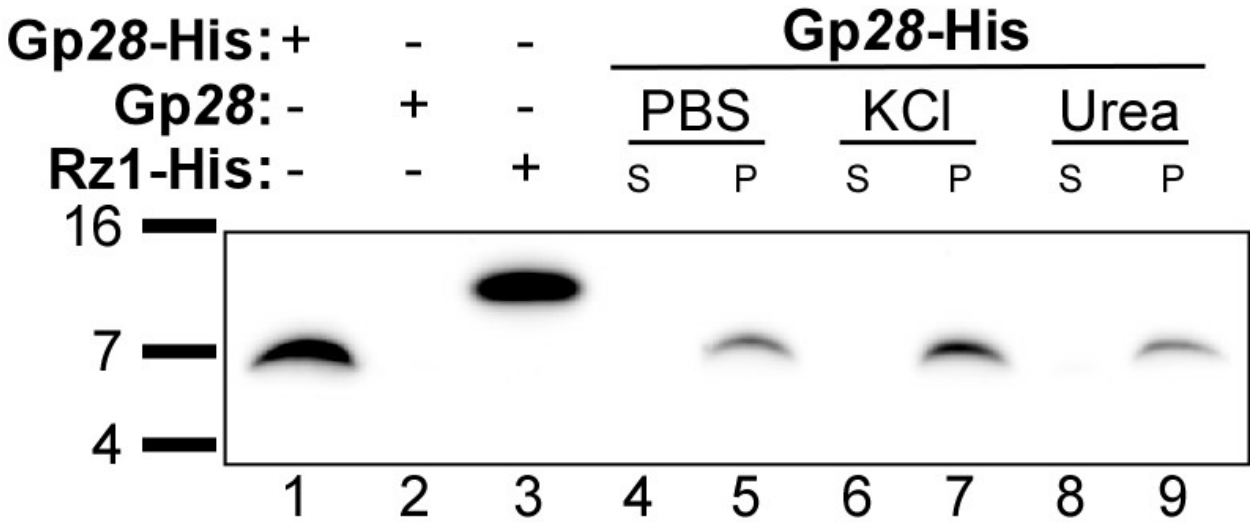

Figure 7. *Salt washes determine the nature interaction with the membrane*. Lysate was collected from MG1655(λ900 $Rz_{am}Rz1_{am}$) containing pRE gp*28*-His. After a series of centrifugation steps exposed to PBS, KCl, or urea, the particulate and soluble fractions were separated and visualized by Western blot.

Fusion to GFP suggests interaction with membrane

To demonstrate an association with the membrane *in vivo*, MG1655 cells producing gp28-sfGFP were imaged prior to lysis (**Figure 8**). The GFP signal appeared in a punctate pattern along the periphery of the cell. This suggests that gp*28*-sfGFP associates with the membrane prior to lysis.

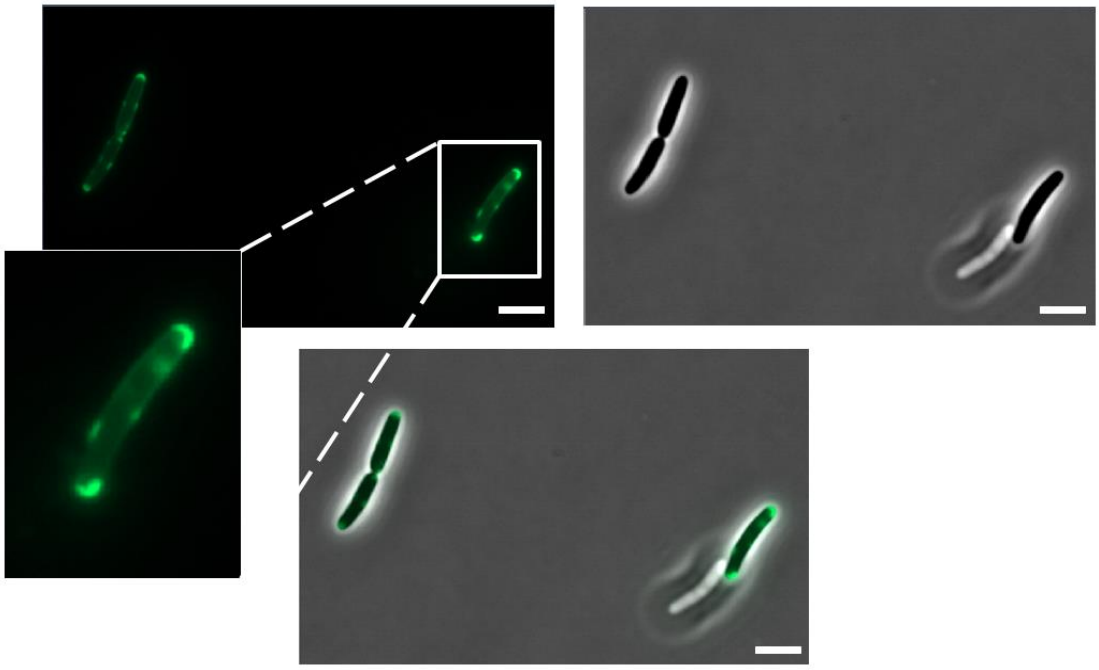

Figure 8. *Microscopy of cells expressing* gp*28-sfGFP*. Fluorescence microscopy and phase contrast microscopy of MG1655 cells containing pRE gp*28*-sfGFP and pQ 40 minutes after induction with 1 mM IPTG.

CHAPTER IV

DISCUSSION

Spanins are the OM-disrupting proteins produced by tailed phages. Nearly all of the molecular information about spanins has come from the study of the prototypical spanins λ Rz and Rzl. However, spanins are a highly diverse and widely conserved class of proteins (10). Recent bioinformatic analysis has shown about 13% of phages do not encode spanins (11). This suggests the probability of a novel mechanism for OM disruption. To address this, we selected ΦKT as a representative spanin-less phage, a coliphage with a simple genome. Here we have identified gp*28*, a new type of OM-disrupting lysis protein.

The disruptin: an antimicrobial peptide produced by ΦKT

Expression of the ΦKT holin, endolysin, and gp*28* in MG1655 resulted in rapid lysis of the culture (**Figure 3**), and gp*28* complements the λ spanin lysis defect (**Figure 4**). These results suggest that gp*28* disrupts the OM. However, gene *28* does not show similarity to spanins. The small hypothetical novel gene is encodes a 56 amino acid protein. This protein has a large net positive charge, and structural predictions indicate an overtly alpha helical character (21). Spanins require an N-terminal transmembrane domain and a lipoylation signal (10), but gene *28* does not contain these features or any other predictors of membrane interaction. Gene *28* would be expected to encode a helical cytoplasmic protein.

We propose that gp*28* is a disruptin: a phage-encoded antimicrobial peptide. Antimicrobial peptides (AMPs) are a structurally diverse class of peptides produced by many different organisms to protect against microbes (22-24). The sequences of AMPs are mostly unique which would prevent the use of a guided BLAST search to identify homologs. The

unifying characteristics of AMPs are a cationic charge along with a large proportional of hydrophobic residues; this amphipathic nature allows for the selective disruption of negatively-charged microbial lipid membranes (22, 23). Gp*28* has a cationic charge and is predicted to be an amphipathic helix. Gp*28* also does not show significant sequence similarity with other proteins, which could indicate the relative importance of general features like small size, charge, and amphipathic structure over the importance of sequence itself.

In addition, gp*28* associated with the particulate fraction after subcellular fractionation, and when fused to sfGFP, gp*28* localized to the periphery of the cell. These results show gp*28* interacts with the bacterial membrane both *in vitro* and *in vivo*. There is also evidence that gp*28* interacts with both the IM and OM. Lysis occurred after the exogenous addition of lysozyme to cells expressing gp*28* without the other lysis proteins. This indicates an interaction with the IM and OM independent of holin activity. This independent interaction with both of the membranes would be expected of an AMP (22).

From these predictions, we propose the following for the function of gp*28* (**Figure 9**). Although the sequence suggests it is a cytoplasmic protein, gp*28* does not require holin hole formation in the IM to disrupt the OM. The susceptibility of cells expressing gp28 to lyse upon the addition of exogenous lysozyme suggests gp28 is prepositioned in the outer membrane prior to the action of the holin. Then the holin releases endolysin into the periplasm. Since the OM has been weakened by gp28 accumulation, lysis occurs after endolysin degrades the PG.

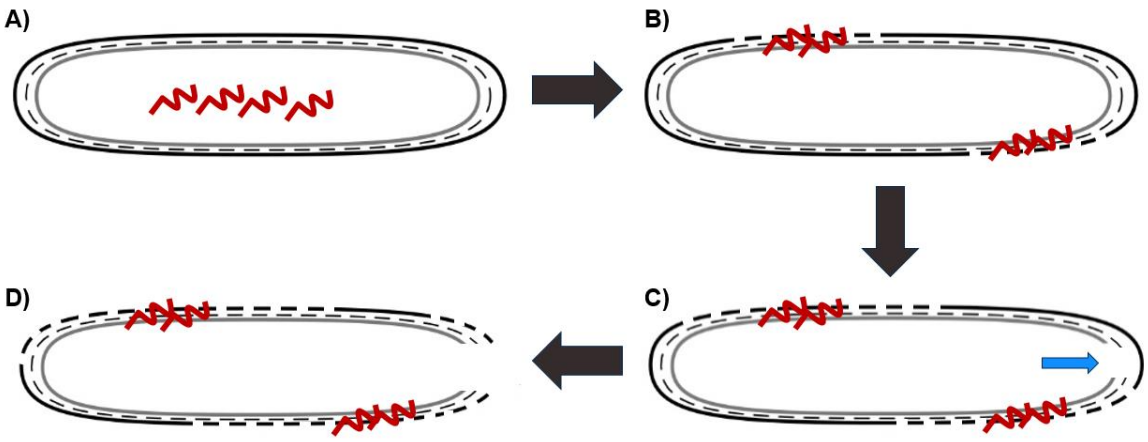

Figure 9. *Proposed model for the mechanism of* gp*28.* A) Gp*28* molecules (red) are produced in the cytoplasm along with the other lysis proteins. B) Gp*28* molecules are positioned in the membrane after expression and prior to holin triggering. As gp*28* molecules enter the membrane, there is a gradual loss of membrane integrity. C) Holin triggering causes a hole in the IM (blue arrow) and allows for the release of endolysin into the periplasm. Both the IM and PG are compromised. D) OM is disrupted by gp*28*, and the cell lyses.

Identifying other disruptins

OM disruption is required for phage lysis, and there are significant number of phages without spanins. The results of this study suggest another mode of outer membrane disruption is mediated by disruptins. If other phages encode disruptins with similar parameters to gp*28*, this type of outer membrane disruptor offers a reduction in genomic space. Gene *28* is roughly a third the size of the lambda spanin genes. In this case, it would be expected that the disruptin mode of lysis would be dominant for most phages, but the opposite is true. Therefore, it may be that there are cases where encoding a disruptin over a spanin may come at a fitness cost to the phage. Lysis timing is especially important to the production of viable phage progeny (2), and the holin is

responsible for the timing of lysis (1, 25). Therefore, an interaction between gp*28* and the IM may threshold the "lysis clock" of the holin, which has been proposed to "tune" itself according to ideal lysis times based on the evolutionary niche. In other words, coding an extra lysis protein that has interaction with the IM could have a severe impact on the fitness of a phage.

Due to the lack of a specific conserved sequence, the identification of other phage AMPs will require unique bioinformatics tools. Future studies aimed toward identifying disruptin genes should be based on general features, such as helicity, charge and size in phages that lack spanin genes.

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