

Research Proposal Summer 2010:
Phage Mutagenesis and the role of SaPII Genes 6 and 7 in Capsid Size Determination

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I. Introduction

Individually, the roles of viruses and bacteria in human disease are well documented. Dangerous bacteria release cell-damaging toxins. Viruses hijack the replication and protein-producing machinery of healthy cells to create progeny phages, and instigate the lysing of healthy cells to enable their escape. Some bacteria, however, have acquired the ability to combine their structural strengths with those of viruses to better distribute their more potent genes.

Staphylococcus aureus is one such species. *S. aureus* is infamous for post-op infections and for its resistance to a growing list of antibiotics (Klebens *et al.*, 2007). As of 2008, it was the cause of over 60% of resistant infections in intensive care patients, and in 2005 the CDC reported that it killed more people than AIDS (CDC, 2008).

S. aureus strains carry a group of genes that produce superantigens, toxins that cause illnesses from food poisoning to Toxic Shock Syndrome (reviewed in (Alouf *et al.*, 2003). Recently, these superantigens, and in some cases the genes in coding for antibacterial resistance as well (reviewed in (Novick *et al.*, 2007)), have been found to be associated with mobile “islands,” or elements of the *S. aureus* genome capable of replication, dispersal, and insertion into a new bacterial cell of the same strain—or even a different species— separate from the transformation of the bacterium’s entire genome (Chen *et al.*, 2009; Maiques *et al.*, 2007).

These islands, or SaPIs (superantigen pathogenicity islands) can’t jump from bacterium to bacterium on their own, however. They require a specific temperate phage to do so (Ubeda *et al.*, 2005; Maiques *et al.*, 2006). Temperate phages, unlike other kinds of phages, do not usually replicate their DNA and lyse their host cell upon infection. Instead, they insert their small genomes into the genome of the bacterium and wait for the cell to come under some kind of attack. Then, in response to trauma to the host, the phage begins replicating its DNA or RNA and creating the protein capsids it needs to transport that genetic information, all of this using the bacterium’s ribosomes, RNAs, and relevant enzymes. The virus has an efficient way of blocking the cell’s genetic information from being processed during this takeover (Tropp, 2008).

SaPIs take complete advantage of this process. Upon infection, the phage derepresses the

SaPI (reviewed in (Novick *et al.*, 2007)). The SaPI then directs the use of viral proteins to form capsids too small for the viral genome, but just right for the SaPI (Poliakov *et al.*, 2008). Within the SaPI region of the *S. aureus* genome are genes for integrases (for integrating into the new host's genome when the SaPI arrives), a terminase subunit (which makes sure the DNA is cleaved into small enough pieces to fit into the capsids), proteins that resemble primases, DNA-binding transcription factors, the superantigens, and an operon that is responsible for formation of the special, smaller capsids and DNA packaging (Ubeda *et al.*, 2007). Many of these genes are very similar to genes that produce the same products or results in viruses, but the SaPI itself is bacterial (Poliakov *et al.*, 2008).

After lysis, SaPI capsids dock on bacterial cell walls and insert the DNA they carry into the cell. The bacterial genome takes up the SaPI, conferring whatever toxic, antibiotic resistant, etc. properties that SaPI might have on the new host (Ruzin *et al.*, 2001). The nature of the genes carried in SaPIs make their study extremely important. Research focused on the interactions between phage and SaPI is a vital subset of that field—if the SaPIs couldn't access the viral proteins for DNA processing and packaging, they would be rendered impotent.

This project will focus on an aspect of that interaction: the small capsids SaPI1 (a SaPI found in the *S. aureus* genome, encoding the toxin that causes Toxic Shock Syndrome (Lindsay *et al.*, 1998)) encodes for, which exclude the DNA of phage 80 α (the phage found to be most successful in derepressing SaPI1 in *S. aureus*). The ultimate goal will be to isolate mutant viruses that, when infecting SaPIs overexpressing the genes directing the formation of smaller capsids, can still package their own DNA and infect new bacteria as if conditions were normal. Mutation in the phages will be encouraged by exposure to UV radiation. These mutants will then be analyzed to determine how SaPIs take control of capsid assembly from wild type phages.

The particular genes necessary and sufficient for SaPI1 capsid formation are referred to as 6 and 7 (based on their location on the SaPI genome, and the direction of transcription) (Poliakov *et al.*, 2008). Gene 6 is believed to control the formation of an internal protein scaffold for the small capsids. Deletion of gene 6 from SaPIbov1 (another, similar SaPI in *S. aureus*) results in an elimination of SaPI procapsids (the necessary precursors to DNA packaging (reviewed in (King *et al.*, 1997)). Gene product 6 (gp6) does not separate from the capsid even after multiple rounds of purification. Electron microscope images of a SaPI1 capsid cross-section reveal an interior texture different than that observed within a typical viral capsid (Ubeda *et al.*, 2007). These findings support the hypothesis that SaPI1 capsids have an internal scaffold dependent on the expression of gene 6.

SaPI1 gene 7's role in capsid formation is less certain. During purification, gp7 easily separates from the capsid, but when gene 7 is deleted, SaPI procapsids do not form (Ubeda *et al.*, 2007). It is therefore reasonable to hypothesize that gp 7 binds some key

capsid protein in such a way as to direct the formation of smaller capsids.

Over the course of this project, SaPI1 genes 6 and 7 will be cloned, transformed into *S. aureus* using a vector that allows for controlled, high expression. Overexpression will hopefully eliminate the observed background phage growth seen with a normal SaPI system. Following ligation and cloning, *S. aureus* will be plated with UV-irradiated phage 80 α , using techniques described below. 80 α mutants generated by this process will be analyzed at a later stage to determine how genes 6 and 7 direct the production of the smaller capsids that allow SaPI1 to proliferate with such enormous success.

II. Progress Report

Over the course of the 2009-2010 academic year, an appropriate overexpression system for SaPI1 genes 6 and 7 was developed. The hypothesis is that overexpression of gps 6 and 7 will eliminate background phage growth, and make the isolation of “SaPI1-resistant” phages possible. Several important factors went into the selection of potential vectors for this purpose: ability to give a high copy number; ability of the inducible promoter to give overexpression of the desired genes; potential toxicity of the inducing conditions for the promoter; ability to carry out cloning in *E. coli* as well as *S. aureus*.

2a. Methods

Four, high copy number, staph-*E. coli* shuttle vector candidates were selected from the literature:

Name of Vector:	Promoter Induced By:	Strain Used:	Selectable Marker For:
pYH4	Anhydrous-tetracycline	RN4220	Erythromycin
pG164	IPTG	SA178-RI	Chloramphenicol
pCN51	CdCl ₂	RN4220	Erythromycin
pJP-B25	Mitomycin-C	RN4220	Erythromycin

Testing the toxicity of the inducing conditions to the bacteria was an important preliminary step. Toxic conditions give sickly lawns, on which it is very difficult to isolate viral plaques. Each vector was therefore screened for toxicity in the same fashion. Each was first electroporated into *S. aureus*. Then, the success of electroporation was established with a quick check. If electroporation was successful, the transformed cells were plated in the inducing conditions. To systems that gave healthy lawns, phage was added to ensure that the inducing conditions did not interfere with 80 α growth either.

2b. Results of Toxicity Screening

pYH4: This plasmid was stored in both RN4220 and the *E. coli* strain DH5 α . Quick check of pYH4 in RN4220 gave a band of the wrong length after multiple trials. The same results were seen with DH5 α . A single digest of the isolated plasmid was attempted, but a band of the right length did not result. Electroporation of pure pYH4 into DH5 α was then attempted—there were no successful electroporations. A graduate of the Christie lab also worked with pYH4, and his notes included a band of the incorrect length as well. Conclusions about this plasmid are that it is either damaged, or incorrectly documented in the literature, but it was ruled out as a potential overexpression system for this project.

pG164: This plasmid was successfully transformed into SA178-RI. This strain was used exclusively with pG164, because of the plasmid's IPTG inducible T7 promoter; SA178-RI has a T7 RNAP. Following transformation, the cells were plated in inducing conditions, resulting in very healthy lawns. Bacteria and phage were then plated together on two different agars: Mueller-Hinton and phage agar. The literature suggested Mueller-Hinton as a better media for IPTG-related systems. The phage grew well (forming many plaques) on both media, but the lawns were thicker with Mueller-Hinton, and so the phage was more readily visible on that media. An important aim of this project is to inhibit background phage growth as much as possible. It is therefore possible that normal phage agar will be a better choice for the mutagenesis stage, despite its less impressive lawns.

pCN51: Transformation was successful. In inducing conditions, the bacteria grew well, but not as well as with pG164.

pJP-B25: Electrotransformation was successful, but the lawns that grew in the inducing conditions were too sickly to be of use in this project, even at very low concentrations of Mitomycin-C.

2c. Conclusions

In both the amount of expression it will give and its permissibility of healthy lawns pG164 is the best candidate for the overexpression of SaPI1 genes 6 and 7. It's use with normal phage agar might further the project's goal of eliminating background phage growth.

III. Summer 2010 Research Plan

The goal for next three months will be to successfully isolate 80 α phage mutants that grow even in the presence of overexpressed SaPI1 genes 6 and 7. The genetic differences between these mutants and wild type phages may provide important insights into the mechanism by which SaPI1 takes over phage machinery

3a. Methods

Primers have already been designed to amplify the region of SaPI1 containing genes 6 and 7. After PCR, the 6 + 7 segment will be ligated into the TOPO cloning vector and inserted into *E. coli* strain DH5 α . This intermediate step is advisable due to known difficulty electroporating with staphylococcus, and so it is a less complicated way of confirming that 6 + 7 have been excised, and a useful means of stable storage.

The 6 + 7 fragment will then be removed from the TOPO vector via restriction digest, and inserted into pG164. Once the insertion is confirmed, then pG164 will be electroporated into a *S. aureus* strain. A quick check of an electroporation colony will be used to confirm that the cloning process is completely.

The next step will be to establish the level of 6+7 expression that completely inhibits phage growth. The IPTG-inducible promoter on pG164 is one element that can be manipulated to create an expression gradient. Once the minimum inhibitory expression level is established, the process of generating and isolating mutants will commence.

A sample of phage 80 α will be mutated using a UV light set up. Multiple trials may be necessary to establish exactly how much UV light is appropriate for the desired level of mutagenesis. The phage and bacteria will then be plated together in inducing conditions (i.e. in the presence of IPTG).

If 6+7-resistant mutants form, they will be isolated for characterization. Characterization will consist of sequencing the mutant genomes and comparing them to the genome of wild type 80 α to determine which altered genes conferred the observed resistance. The products of these genes will then be earmarked for further studies on phage-bacterium protein interactions.

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