

Research Proposal Summer 2009: *The role of SaPII gene products 6 and 7 in capsid size determination*

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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 (Klevens *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 “islands,” or isolated areas 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 immediately 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 some trauma to the host cell, 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 unrepreses 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 unrepressing 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* strain SA178-RI using a vector that allows for controlled, high expression. The resulting lawns will then 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 which allow SaPI1 to proliferate with such enormous success.

Methods and Materials

A. Determination of a viable shuttle vector for expressing SaPII genes 6 and 7

Expression of genes 6 and 7 must be high, but tightly controlled. To accomplish this, a cassette-based shuttle vector will carry the two genes into *S. aureus*, and will be manipulated to produce the desired level of expression.

A cassette-based shuttle vector is a vector consisting of a multiple cloning site, a thermosensitive replicon (from staphylococcus), two selectable markers (one from *E. coli*, and one from staph), a segment of phage DNA that allows for effective transduction, a segment of SaPII DNA that allows for integration at a specific site on *S. aureus*'s genome, and a promoter that can be induced to commence transcription. Each of these elements, these cassettes, has been inserted into the vector using a pair of restriction sites, which means they can be removed and/or replaced with some other useful gene (Charpentier *et al.*, 2004).

The multiple cloning site allows for copies of the vector to be made. The thermosensitive replicon, in this case, increases the level of replication at higher temperatures and decreases it at lower temperatures. Having a selectable marker for both *E. coli* and *S. aureus* means the vector can be cloned in either species. The phage DNA is especially important with Gram-positive bacteria, like *S. aureus*, which have thick cell walls, and do not take up vectors as easily as a Gram-negative bacterium, like *E. coli*.

The vector used to clone and express 6 and 7 must be carefully selected and tested prior to cloning, because some of the inducible promoters operate using chemicals that could, in conjunction with the very high levels of expression, be toxic to the cells. In order for phage mutant plaques (blank areas on the plate where a phage has killed bacteria) to be distinguishable, the bacteria must form a healthy lawn. Thus, a vector selection process is the first step in cloning genes 6 and 7.

A1. Expected Results and Troubleshooting

A potential vector, with an IPTG-inducible promoter, has already been selected by the PI. This vector, pJP-B25, has already been transformed into SA178-RI, but whether or not it inhibits the growth of a healthy lawn has yet to be determined. IPTG is a stand-in for allolactose in the *lac* operon system, and is similarly useful in initiating the transcription of artificially assembled systems like pJP-B25. If a lawn does form, pJP-B25 will be used to clone the desired genes. If not, the process of transforming SA178-RI (described below) will have to be repeated with a different candidate vector. Other options include a cadmium-inducible promoter, and one that induces an SOS response using mitomycin C.

B. Amplification of Genes 6 and 7

Genes 6 and 7 will be amplified by PCR, using primers that contain the restriction sites necessary for insertion into a shuttle-vector.

B1. Expected Results and Troubleshooting

As with all PCR, there is a risk of contamination by foreign DNA in the sample. Gel electrophoresis can be used to quickly ascertain the purity of the product. If bands of DNA, other than that of the anticipate restriction sites + gene 6 + gene 7 band, appear, the PCR will have to be repeated until a pure product is obtained.

C. Electrotransformation of vector containing Genes 6 and 7 into SA178-RI

Electrotransformation is a process in which bacteria are induced to take up a desired vector by experiencing an electrical pulse. The cells must first be rendered electrocompetent using a series of cold glycerol washes.

C1. Expected Results and Troubleshooting

The process of creating electrocompetent cells is not always successful. If, after the cells have been processed, a plated sample fails to form colonies, more cells will need to be made electrocompetent before electroportation can take place. In addition, the particular strain being used for this project, SA178-RI, is difficult to transform, which could necessitate multiple rounds of electroportation with fresh cells.

After transformation takes place, a miniprep to isolate the vector will need to be carried out to ensure that the plasmid was successfully taken up by the bacteria. Once the DNA has been removed from the cells and eluted in water, it can be run on a gel to establish a) that the desired product is present, and, b) in what concentration. During the miniprep, damage to the DNA might necessitate centrifuging out another pellet of transformed cells and isolating the DNA once more to get a clear band on the gel.

E. Isolation of mutant 80 α strains

Once a healthy lawn has formed, the promoter in the selected vector will be induced to begin transcription of SaPI1 genes 6 and 7. The conditions experienced by the bacteria will favor high expression, as dictated by the thermosensitive replicon. Phage 80 α will be subjected to UV radiation, then introduced to the plates, and infection of the *S. aureus* cells will take place. Because SaPI1 genes 6 and 7 are responsible for the formation of capsids too small to package phage DNA, only phages with mutations that prevent capsid-size determination by the SaPI1 genes will successfully spread and form viral plaques.

E1. Expected Results and Troubleshooting

Given the considerable mutagenic properties of UV radiation, it is reasonable to expect that at least some functional mutants will be isolated by this process.

Successful mutation by this mechanism has been carried out in T4—a bacteriophage involved in a system used to model 80 α -SaPI1 interactions (Drake, 1966).

Timeframe and Implication

This project should see completion by August 1, 2009. Many of the procedures involved take one or two days of lab work, and this stage requires no sequencing or analyzing of large data sets.

Once the mutants have been isolated, however, their genomes will be sequenced, the gene products crystallized, and the findings compared with the 80 α wild type. Such a comparison could produce a model illustrating how, on a molecular level, SaPI1 determines capsid size, and therefore how the transduction of the toxins SaPI1 contains might be interrupted. Given the high level of relatedness between the SaPIs of *S. aureus* (Ubeda *et al.*, 2007), a model of this kind has the potential to illuminate not only the mechanism in SaPI1, but those found in many other SaPIs as well.

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