Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter know, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Kirsten Baecher 4/12/11

Use of a synthetic riboswitch to determine the effects of Erc on the regulatory element CovR and associated virulence genes in *Streptococcus pyogenes*

by

Kirsten Baecher

June R. Scott
Adviser

Department of Biology

June R. Scott

Adviser

Arri Eisen

Committee Member

Kathleen Campbell

Committee Member

4/20/11

Use of a synthetic riboswitch to determine the effects of Erc on the regulatory element CovR and associated virulence genes in *Streptococcus pyogenes*

By

Kirsten Baecher

June R. Scott

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2011

Abstract

Use of a synthetic riboswitch to determine the effects of Erc on the regulatory element CovR and associated virulence genes in *Streptococcus pyogenes*

By Kirsten Baecher

The human pathogen *Streptococcus pyogenes* (Group A Streptococcus, GAS) is responsible for various diseases, which range in severity from minor self-clearing infections like those of the throat and skin to severe, life-threatening diseases such as necrotizing fasciitis. The severity of an infection is mediated by the expression of virulence factors encoded in the S. pyogenes chromosome. One mechanism by which expression of these factors (as well as other genes) is regulated is the 2-component regulatory system, CovR/S. CovR is a response regulator protein that, when bound to DNA, represses transcription. It has been demonstrated that in addition to being under control of its own promoter, CovR transcription is also promoted by the upstream Erc promoter. The role of the Erc protein in this pathway, however, remains unclear. In this investigation, I worked towards construction of a plasmid that could be used to control Erc translation via a synthetic riboswitch, which would ultimately be used in the host chromosome for assays of expression of elements under CovR control, at different levels of Erc expression. I discuss challenges that were presented during the construction of the plasmid and their molecular basis, as well as potential strategies for overcoming them in future work.

Use of a synthetic riboswitch to determine the effects of Erc on the regulatory element CovR and associated virulence genes in *Streptococcus pyogenes*

By

Kirsten Baecher

June R. Scott

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2011

Acknowledgements

Special thanks to Barbara Froehlich and Jeff Freiberg for preparation of template plasmids, and for assistance and advice throughout the investigation. Also, thanks to Gordon Churchward for insight into the molecular aspects of the investigation.

Table of Contents

Index of Figures

I. Purpose

The purpose of this project is to investigate the effects of a protein known as Erc (or Duf177) on the regulatory element CovR and the genes under CovR control in *Streptococcus pyogenes*. Expression of the Erc gene will be directly controlled via a synthetic riboswitch. The construct will be inserted into the *S. pyogenes* chromosome and once the effects of the riboswitch on Erc are characterized, controlled expression of Erc will determine Erc's effects on the expression of CovR and of virulence factors under its control.

II. Introduction

The human pathogen *Streptococcus pyogenes* (Group A Streptococcus, GAS) is responsible for various diseases, which range in severity from minor self-clearing infections like those of the throat and skin to severe, life-threatening diseases such as necrotizing fasciitis. GAS infection can result in sequelae such as rheumatic heart disease and glomerulonephritis, depending on the pattern of expression of virulence factors. *S. pyogenes* is able to adjust its gene expression when it senses stressors such as extreme pH or high temperature, thereby allowing it to adapt swiftly to harsher conditions. One pathway by which this bacterium is able to rapidly alter gene expression in response to changes in the environment involves the CovR (control of virulence; sometimes also called CsrR) transcriptional regulator, which in the absence of environmental stress represses approximately 15% of the genome. Many of the repressed sequences are genes encoding virulence factors for GAS, as well as promoter regions for additional virulence factors including streptococcal DNase and streptolysin S (Dalton and Scott, 2004). CovR expression is mediated by a negative feedback loop, wherein CovR represses its own promoter which is located upstream.

In most bacteria, the stress response system involves regulation by sigma factors, which alter RNA polymerases to facilitate transcription of genes not usually expressed. In GAS however, there are no sigma-like factors; instead, CovR is part of the two-component regulatory system that senses and responds to stressors in the environment. It has been shown that another factor, CovS, is the sensor component necessary to relieve gene repression by CovR to allow gene expression of virulence factors and permit GAS growth in stressful environments (Dalton and Scott, 2004). The CovS/CovR regulatory system belongs to the OmpR family of proteins, which consists of a number of different two-component regulatory systems that share some basic putative characteristics. In general, the effector protein (CovR or OmpR) forms a two-domain protein upon translation; one (larger) domain is the signal receiving domain, and the other is the DNA-binding domain. Upon (reversible) phosphorylation by the sensor protein, the effector protein forms a homodimer which exhibits higher affinity to DNA, affecting transcription of downstream sequence (Fig. 1). This interaction has not been biochemically shown in the CovS/CovR system; in fact, it has been suggested that while CovS acting as a kinase is responsible for phosphorylation of CovR to CovR-P, it is also capable of inactivation of CovR-P by dephosphorylation (Churchward).

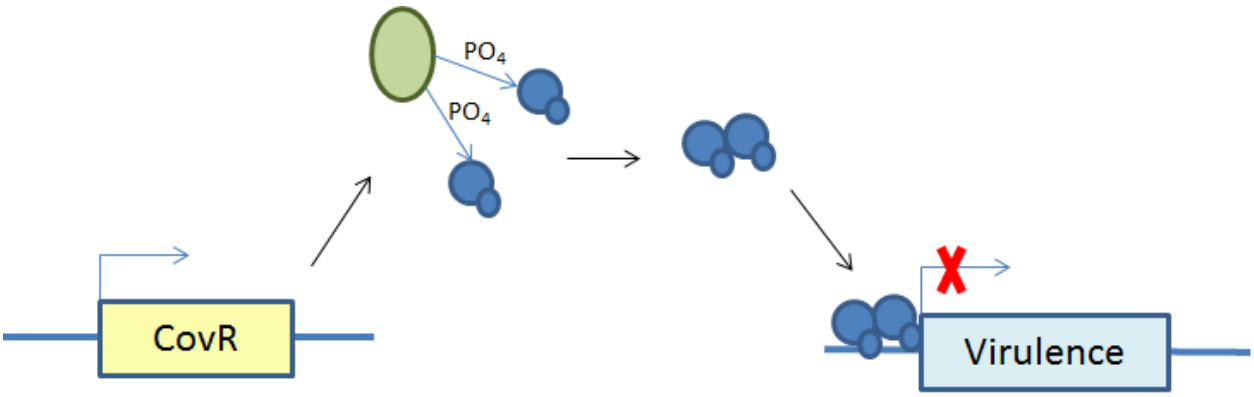

Fig. 1: CovS/CovR 2-component regulatory system. Kinase, presumably CovS (shown in green), phosphorylates CovR (blue) monomers, causing dimerization and facilitating DNA binding.

Another protein, Erc, has also been experimentally implicated in the regulation of CovR. Like the upstream CovR promoter, the Erc promoter is repressed by the CovR protein (Froehlich and Scott, unpublished); furthermore, when Erc is transcribed there is downstream transcription of CovR (Fig. 2). Although this demonstrates the role of the Erc promoter, the effects of the Erc protein and its interaction with other components of the stress-response system are unknown. Because phenotypic differences in CovS/CovR regulation and expression of virulence factors between group A streptococcus (GAS) infections can often determine the severity of infection and level of invasiveness (Sumby), it is likely that Erc may also be involved in the differences between self-limiting and life-threatening infections. Elucidating this relationship may lead to avenues for new therapeutic strategies; however, although Erc does not have a homolog in the human body (it has limited partial homology to some structurally relevant proteins, but none greater than 56%), it is highly conserved across many bacterial species. Attempting to manipulate levels of this protein may have a broad effect on organisms with which humans exist symbiotically, such as the gut flora.

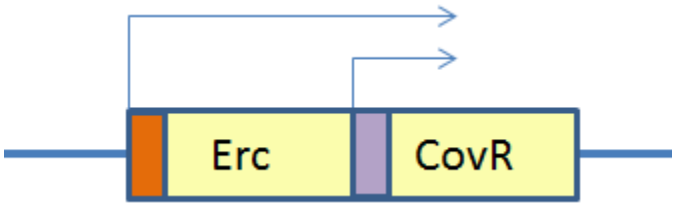

Fig. 2: *erc* and *covR* gene positions. *covR* is under control of both *erc* promoter (orange) and its own *covR* promoter (purple).

In order to control translation of the Erc transcript (rather than controlling transcription) thus ensuring that the observed effects in vivo are due to the protein product and not to transcription, this experiment employs another bacterial genetic control mechanism, the riboswitch. A tool that is conserved across all bacteria, the riboswitch is a sequence of mRNA that takes a characteristic form upon transcription from the DNA template, wherein the ribosome

binding site of the transcribed gene is sequestered thus inhibiting translation. The switch is able to respond directly to ligands in the cell to control *cis* elements of mRNA (Stormo). When the riboswitch is exposed to its ligand, the ligand binds to a particular set of bases (the aptamer) of the mRNA and induces a conformational change which exposes the ribosome binding site (RBS) and allows translation. Synthetic riboswitches have been designed which are optimally responsive, specifically to activation by the small molecular ligand theophylline (Topp et al). In particular, "Riboswitch E" (used in this experiment because of the high efficacy it has demonstrated in *S. pyogenes* plasmids) was derived from another switch ("Riboswitch D") which was selected from a library of theophylline-dependent riboswitches with randomized RBS sequence between the aptamer and the start codon, riboswitch D is optimally efficient in *Acinetobacter baylyi*, *Acinetobacter baumannii*, *Agrobacterium tumefaciens*, and *Mycobacterium smegmatis*. Switch E was then generated through the insertion of sequence AGG into the RBS of switch D (Fig. 3).

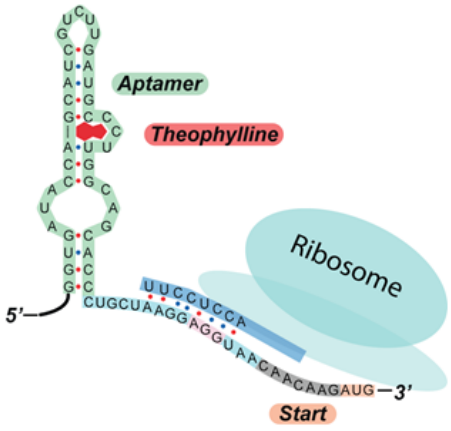

Fig. 3: Structure of activated riboswitch E (adapted from Topp, et al). Aptamer (green); RBS (blue); AGG insertion (pink); star codon (peach).

Dose response profiles in *E. coli* and *S. pyogenes* demonstrate that when switch E is inserted 3' to the promoter and the 5'-UTR (untranslated region) of a reporter gene in a plasmid, there is strong dependence on theophylline in order to allow translation of downstream mRNA. Riboswitch responsiveness was measured in *S. pyogenes* via the reporter gene β-glucuronidase [Gus]; switch E was the only switch that showed significant levels of control over the reporter gene, with very high Gus

translation when ligand was added and a high activation ratio of signal to background expression (Topp et al).

It is important to note that because riboswitch control of gene expression takes place post-transcriptionally, methods such as PCR or northern blots would not provide any information about the activity of the riboswitch. In order to detect expression of protein controlled by the riboswitch in this experiment, a genetic sequence (or "tag") is inserted into the gene so that upon translation, the resulting amino acid sequence is recognizable by an antibody designed against that specific tag. This particular tag, ("Flag" epitope tag) has been used successfully to identify *S. pyogenes* gene products (Baecher, Scott lab) via western blot.

The goal of this project is twofold: first, to assess the efficacy of the synthetic riboswitch's ability to control translation in a chromosome, rather than in a plasmid; and second, to elucidate the effects of Erc expression on the CovR regulatory system by controlling Erc expression and measuring changes in transcription of other genes under CovR control. The first objective will be accomplished by inserting the Flag epitope tag into the *erc* gene sequence, with riboswitch E immediately upstream of the *erc* start codon. This genetic construct will be generated in a plasmid and then recombined into the *S. pyogenes* chromosome. A dose response profile to theophylline will be generated based on Flag translation as measured by western blot. The second objective will be accomplished by recombination of a different plasmid (identical to the first described plasmid, but without the Flag insertion) into the *S. pyogenes* chromosome, and then at various levels of Erc translation (according to the information generated by the Flag-tagged Erc) quantitative reverse-transcriptase PCR (QRT-PCR) will be used to characterize the levels of transcription of the CovR regulon.

III. Methods

Bacterial strain and media information: for cloning in *E. coli*, strains TOP10 (Invitrogen) and XL10 Gold (Stratagene) were used. The *S. pyogenes* strain is MGAS 2221 (serotype M1). All cultures were grown in LB/kanamycin (50 ug/ml) at 37˚C overnight unless otherwise specified.

DNA manipulation information: All restriction digests were performed with restriction enzymes from New England Biolabs (NEB) according to manufacturer's suggestions at 37˚C for 1 hour unless otherwise specified. All ligations were performed with Quick Ligase (NEB) according to manufacturer's instructions. All PCR reactions (except for Quikchange reactions) used Platinum Taq High Fidelity (Invitrogen) with programs designed according to manufacturer's suggestions. All agarose gel electrophoresis (AGE) was carried out in 0.8% agarose in tris acetate EDTA (TAE) buffer at 120 volts with ethidium bromide to visualize the samples.

IV. Results

Plasmid construction

1. Overview

PCR and ligation at restriction enzyme sites were used in attempts to engineer 2 constructs in an *E. coli* vector (pCR-XL, Invitrogen) containing: (1) the erc promoter with a synthetic riboswitch inserted after it, (2) the Erc gene sequence, regions of (3) upstream and (4) downstream homology to the *S. pyogenes* chromosome, and (5) a gene for antibiotic resistance, specifically to tetracycline (*tetM*). One of these constructs, pEU8491, also has a FLAG epitope tag

(DYKDDDDK) (Sigma) inserted at the 5' end of the Erc gene, 6 base pairs after the ATG start codon, making it recognizable by monoclonal anti-FLAG antibody (Sigma). The plasmid without the tag is pEU8492.

Each plasmid was to be synthesized by joining 3 smaller sequences: the "upstream region" (pink), *tetM* (blue), and the "downstream region" (yellow) (Fig. 4).

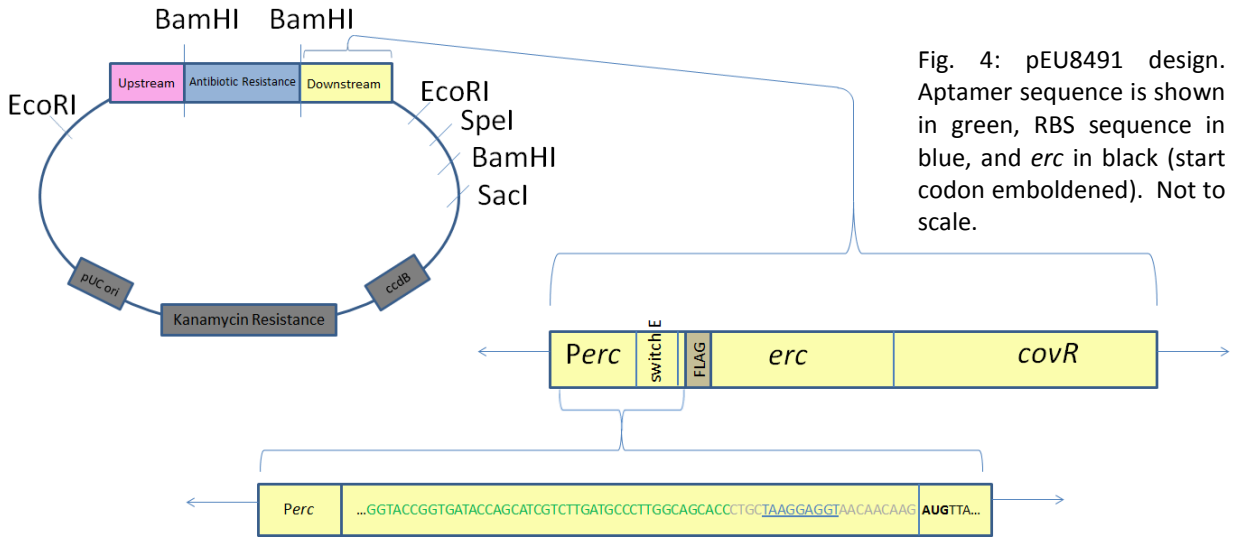

Fig. 4: pEU8491 design. Aptamer sequence is shown in green, RBS sequence in blue, and *erc* in black (start codon emboldened). Not to scale.

The "upstream region" was derived via PCR amplification of the relevant region of MGAS2221 chromosomal DNA and includes *hptX* (*S. pyogenes* heat shock protein). The complete "downstream region", containing the *erc* promoter, riboswitch, *erc* and *covR* sequences was previously constructed in 5 different plasmids (pEU8473, pEU8474, pEU8475, pEU8481, pEU8482; Froehlich)), 2 of which contain the Flag tag inserted into *erc*. The riboswitch sequence (riboswitch E) was obtained from pCKR4 and inserted immediately 3' to the P*erc* 5' UTR. The

erc start codon is located immediately downstream of the riboswitch E-specific ribosome binding site sequence (AAGGAGGT) and the conserved CAACAAG (Fig. 4).

2. pEU8492 Construction

The first attempt at creating the plasmid without the flag tag (-FLAG) for recombination was to PCR amplify the *tetM* gene from existing constructs (pEU8481 and 8482), then use overlapping PCR to fuse the *tetM* gene and the upstream region PCR product, using primers which introduce a restriction enzyme site (XbaI or NotI) on each end of the fragment. The product would be digested with restriction enzymes and inserted by ligation into the plasmids with the –FLAG downstream region (pEU8473, 8474, and 8475) (Fig. 5).

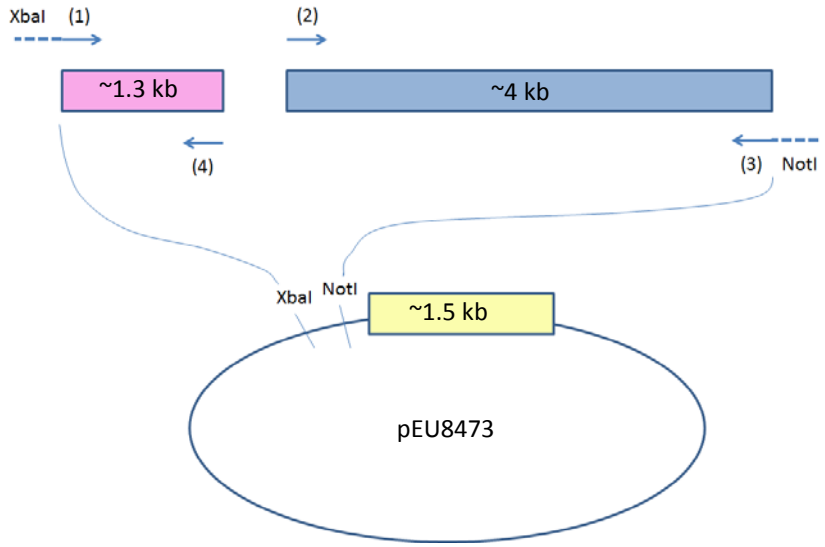

Fig. 5: Construction plan for pEU8492. Upstream fragment was amplified by primers (1) and (4); *tetM* by (2) and (3); fusion by overlapping PCR with (1) and (3). The fragment would then be inserted into pEU8473 by digestion and ligation.

Although each set of first round PCR reactions yielded product of the correct size (~4 kb for *tetM* and ~1.3 kb for upstream region), the overlap of the two fragments did not yield any overlap product (Fig. 6).

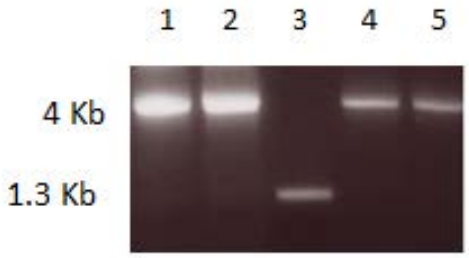

Fig. 6: Agarose gel electrophoresis of PCR products. Lanes 1-2 are the products of the [failed] overlapping reaction. Lane 3 is the upstream region. Lanes 4-5 are *tetM*.

The second attempt towards engineering the target plasmid was to join the downstream homology region and *tetM* by overlapping PCR and then to insert the resulting fragment into the pCR-XL Topo vector (Invitrogen). Following this, the upstream region would be inserted either by restriction enzyme site ligation or by overlapping PCR with the downstream/*tetM* fragment and subsequent insertion into vector (Fig. 7).

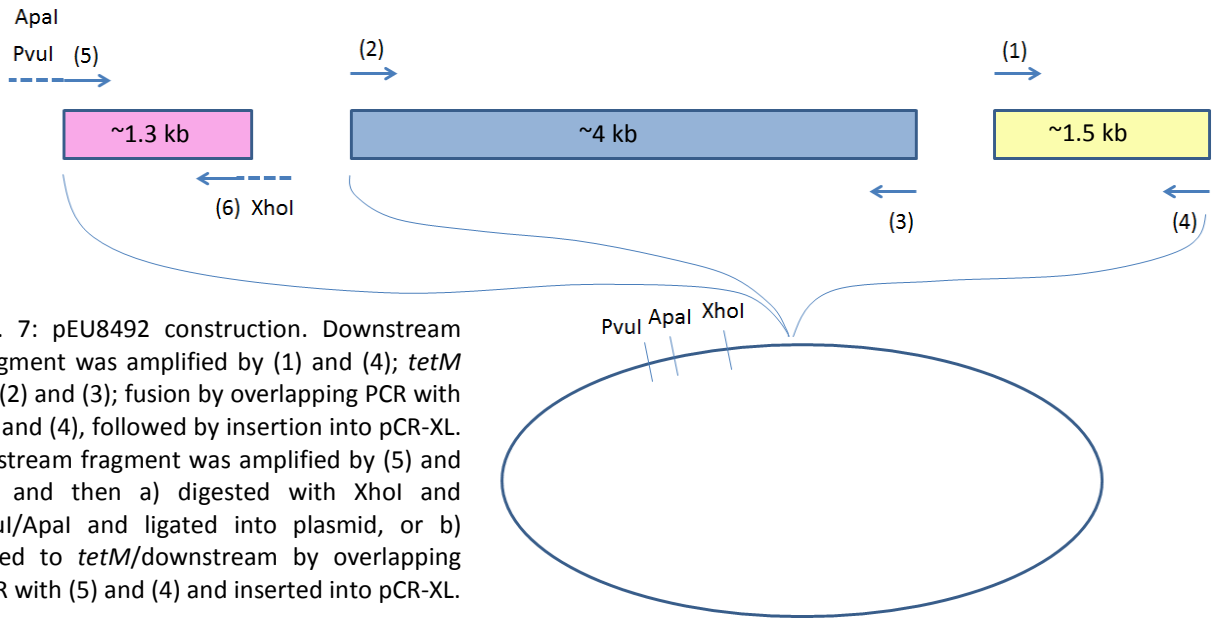

Fig. 7: pEU8492 construction. Downstream fragment was amplified by (1) and (4); *tetM* by (2) and (3); fusion by overlapping PCR with (2) and (4), followed by insertion into pCR-XL. Upstream fragment was amplified by (5) and (6) and then a) digested with XhoI and PvuI/ApaI and ligated into plasmid, or b) fused to *tetM*/downstream by overlapping PCR with (5) and (4) and inserted into pCR-XL.

The first step of this approach (fusing the downstream –FLAG homology and *tetM*) yielded the desired product, confirmed by selection with LB/tetracycline (10 ug/ml) and restriction analysis (BamHI) (Fig. 8). However, subsequent attachment or insertion of the upstream homologous region was unsuccessful by both overlapping PCR and restriction digest (XhoI and PvuI or ApaI) followed by ligation.

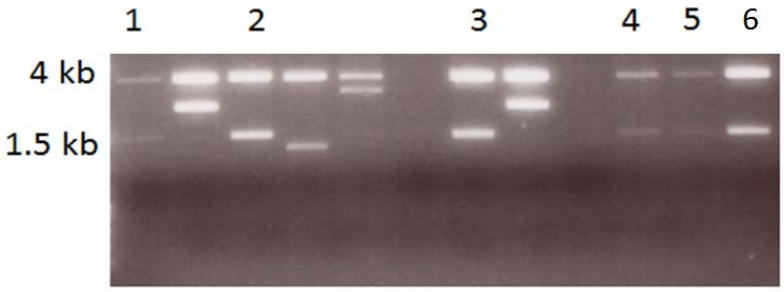

Fig. 8: AGE of recombinant plasmids after transformation into *E. coli*. Lanes 1-6 show band at 4 kb (corresponding to *tetM*) and 1.5 kb (corresponding to the downstream region).

The third attempt was designed to circumvent the issues that were presented with attaching the upstream region with the *tetM* gene. The upstream region from MGAS2221 and the downstream regions from –FLAG (constructs from attempt 2) would be amplified by PCR and overlapped, using primers which would add a BamHI site at the overlap locus (Fig. 10) This fragment was inserted into pCR-XL Topo vector, then following transformation (into OneShot TOP10 chemically competent cells) was analyzed by restriction digest and sequenced.

Sequencing revealed that the two fragments had been successfully joined but that there was a point mutation at the n-terminus of the *covR* gene in the start codon. Two approaches were utilized to fix this mutation: the first was to use PCR for site-directed mutagenesis (Quikchange II XL, Stratagene) with primers designed to correct the mutation (C→T). Transformation by the PCR products (into XL10-Gold ultracompetent cells, Stratagene) yielded no colonies. The second approach was to re-amplify the downstream region directly from the parent plasmids pEU8473, 8474, and 8475. These fragments were overlapped again with the upstream fragment, and the product inserted into the vector; the resulting transformants were analyzed by restriction digest.

The samples that appeared to have the correct size inserts then were treated to remove the vector BamHI site downstream of the insert (to ensure that digest by BamHI for drug insertion will allow insertion only between the upstream and downstream regions, and not in the vector). Three approaches were used to remove the site: the first was to digest the plasmid with restriction enzymes that have sites closely adjacent to BamHI (SacI and SpeI) (Fig. 1a), then to blunt the ends (Quick Blunting kit, NEB) and ligate them together to excise the BamHI site. This ligation

did not result in any target transformed plasmids in TOP10. The second approach was to again use site-directed mutagenesis with the appropriate primers ((3) and (8), Fig. 10), and the third was to cut both the insert and the plasmid pEU8351 (a derivative of pCR-XL vector with deletion of the BamHI site of the polylinker; Freiberg) with EcoRI, purify the vector by agarose gel electrophoresis and extraction (Quickspin kit, Qiagen), and ligate the insert into the vector. Only transformations (in XL10) of the Quikchanged plasmid resulted in the desired construct. The plasmids were isolated and analyzed by restriction digest (Fig. 9). This construct is now called pEU8490.

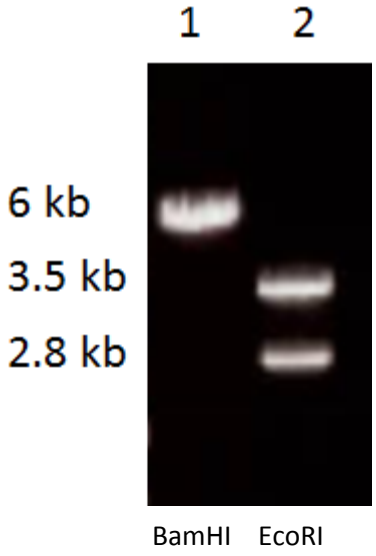

Fig. 9: AGE of pEU8490. Plasmid in Lane 1 cut with BamHI. Plasmid in Lane 2 cut with EcoRI (3.5 kb band corresponds to pCR-XL vector; 2.8 kb vector corresponds to downstream + upstream region).

The final step in the construction of pEU8492 was to insert the *tetM* gene at the remaining BamHI site. The *tetM* gene was isolated from pEU8481 template by PCR, and then digested with BamHI along with the plasmids. The two pieces were ligated together and used to transform TOP10 cells; however, no transformants grew. In order to sidestep the issues that the *tetM* gene has presented, the smaller chloramphenicol resistance gene (Cm^R) was amplified from pEU8368 using primers which added BamHI sites on both ends, and inserted into the –FLAG plasmids in

the place of *tetM*. When the resulting colonies were selected by incubation in LB/Chloramphenicol (40 ug/ml) broth, no growth occurred; the procedure was repeated but with digestion of Cm^R by restriction enzyme for a 300% increase in incubation time, and with treatment of the vector (-FLAG construct) with shrimp alkaline phosphatase prior to ligation to prevent self-ligation. The transformations in TOP10 were selected by LB/Cm (40 ug/mL) agar, but still did not yield any colonies.

3. Construction of pEU8491

In order to add the Flag tag to pEU8492, 2 different approaches were employed: (1) addition of the Flag tag by PCR (using primers (4) and (7), Fig. 10) followed by overlapping the two resulting PCR fragments (using primers (1) and (6), Fig. 10), as well as (2) Quikchange PCR, but neither produced any colonies following transformation in either XL10 or TOP10.

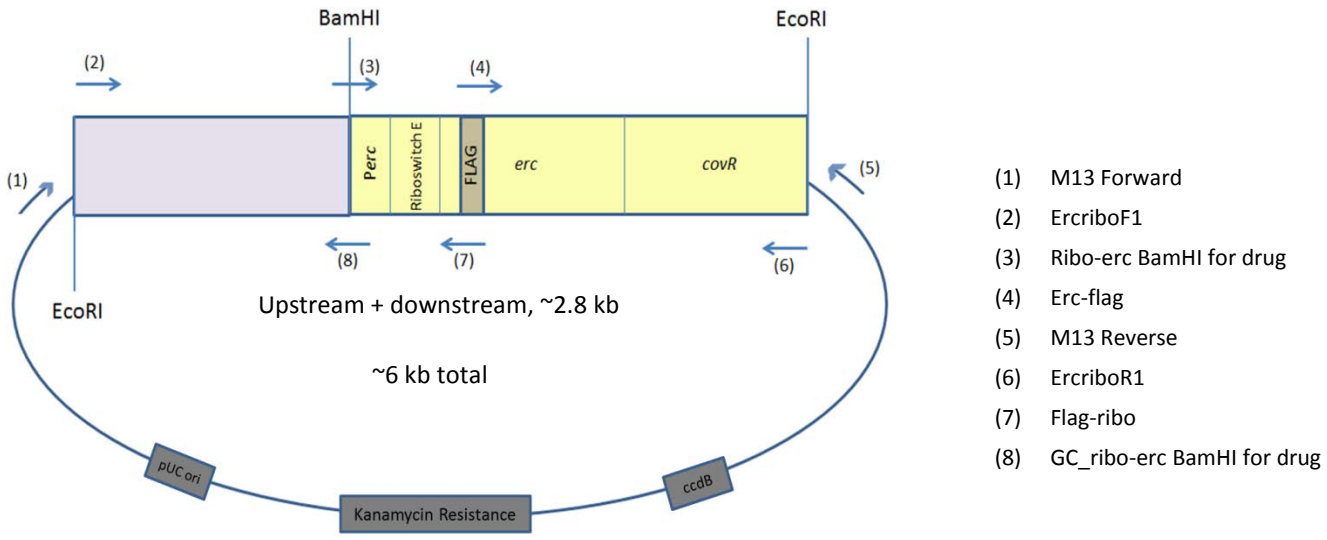

Fig. 10: Map of target plasmid and primer anneal sites used its construction.

V. Discussion

1. -FLAG construction completed, without insertion of antibiotic resistance gene.

pEU8490 contains the upstream region of homology derived from MGAS2221 and the "downstream region" as depicted in Fig. 2, with only one BamHI site in the entire plasmid, located between the homologous regions. This site is intended for use as a locus for insertion of antibiotic resistance gene (or other gene of interest) flanked by BamHI sites. The construct has been confirmed by restriction analysis (Fig. 8) and sequencing.

2. **pEU8490 contains two uncorrected mutations** of unknown impact on gene activity.

The first mutation is from a tyrosine to an adenine in the P*erc*-riboswitch overlapping primer sequence, which may or may not affect promoter activity in vivo.

The second is a guanine to adenine change in the aptamer region. This mutation is very likely to affect the riboswitch's ability to bind theophylline, as this guanine pairs with a cytosine upstream on the strand thus affecting switch structure, and is conserved in all of the synthetic switch types developed by Topp et al.

3. ***E. coli*** **resistance to CovR expression.**

It is likely that the problems with antibiotic resistance gene insertion into the plasmid are due to the difficulty surviving that *E. coli* experiences when CovR expression is induced. It has been shown that *E. coli* rarely can be engineered to express CovR; it is probable that translation of CovR interferes with an endogenous two-component regulatory system, interrupting the sensor pathway and causing inappropriate transcription.

Experimental design attempted to account for this by using less than half of the whole *covR* sequence in the plasmid (101 amino acids versus the complete protein 228; almost the entire signal receiving domain is included, but none of the sequence for the DNA binding domain is present). However, the prevalence of the cytosine to thymine point mutation at the start codon of the gene (eliminating transcription) indicates that shortening the sequence does not adequately alleviate the host's resistance to CovR expression. Populations of *E. coli* containing that mutation, which are not expected to have translated *covR*, outcompeted and overtook any populations that were burdened with expression of even partial CovR protein.

This partial CovR protein, because it contains almost the entire signal receiving domain, is likely to be capable of interacting with endogenous sensory proteins and, upon phosphorylation by that protein, may form a heterodimer with an endogenous effector protein. This interference of CovR into the resident *E. coli* sensory pathway could be detrimental to the bacterium in a number of ways; for example, the heterodimer might either over-induce or inactivate transcription of important genes. Specifically, it could alter the efficacy of the endogenous effector protein binding to DNA. In the event that the heterodimer increases DNA binding affinity, the downstream transcript may be extensively repressed beyond the normal

requirements of the cell, leading to deficiency of the encoded component. If the opposite occurs, and heterodimer formation decreases the response regulator's affinity to DNA, there will be inefficient inhibition of the target gene in response to a given stressor.

4. Future Directions

Due to the difficulties presented by the expression of CovR in *E. coli*, plasmid construction might be made less problematic by excluding the *covR* sequence from the region of downstream homology. The length of the *erc* sequence (799 base pairs) is long enough to facilitate crossover into the *S. pyogenes* chromosome. The upstream region (*hptX*) may also be shortened to a similar length in order to prevent the likelihood of a single-crossover event.

Prior to recombination into *S. pyogenes*, the Flag epitope tag must be inserted at some site in *erc*. Although this construct should be able to accept insertion of the Flag epitope tag by PCR, none of the efforts to this end have been successful. It may be beneficial to future work on construction of these plasmids to design primers which insert the Flag sequence further downstream in *erc*, or amplify by PCR from the pEU8481, 8482 template which already has the Flag insertion, and to insert that downstream fragment in the place of the downstream fragment in pEU8490.

Upon successful construction of pEU8491, the plasmid sequences may be recombined into the S. pyogenes chromosome and subsequently assayed for genetic control as described in the introduction. Erc may be a promoter for CovR transcription (as evidenced by *covR* transcription when exposed to Erc). Therefore it is likely that at high levels of theophylline (causing erc

activity to be induced by the riboswitch), there will be strong repression of virulence factors. For example, some proteins under CovR control (such as streptokinase, streptococcal DNase and streptolysin S) (Dalton and Scott, 2004) will be expressed when not exposed to theophylline, but will be underexpressed or not at all when the ligand is added. These differences could be quantified in terms of transcription by measuring the mRNA for those proteins first by microarray analysis (because it is relatively high-throughput), and then more specifically by QRT-PCR; or, if there are antibodies available to the protein product, translational implications could be measured by western blots which could identify the presence and give a semi-quantitative measurement of protein concentration. These translational effects, particularly for the toxins, could also be measured by enzymatic activity assays.

VI. References

Churchward, Gordon. 2007. "The two faces of Janus: virulence gene regulation by CovR/S in group A streptococci." Mol Microbiol. Apr;64(1):34-41 DOI: 10.1111/j.1365-2958.2007.05649.x

Dalton TL, Hobb RI, and Scott JR. 2006. "Analysis of the role of CovR and CovS in the dissemination of Streptococcus pyogenes in invasive skin disease." Microb Pathog. 40(5):221-7. Epub 2006 Mar 20.

Dalton TL and Scott JR. 2004. "CovS inactivates CovR and is required for growth under conditions of general stress in *Streptococcus pyogenes*." J. Bact DOI: 10.1128/JB.186.12.3928-3937.2004

Gusa AA, Froehlich BJ, Desai D, Stringer V, and Scott JR. 2007. "CovR Activation of the Dipeptide Permease Promoter (P*dppA*) in Group A Streptococcus." J. Bact DOI:10.1128/JB.01036-06

Stormo, Gary D. 2003. "New Tricks for an Old Dogma: Riboswitches as *cis*-Only Regulatory Systems" Molecular Cell, Vol 11 Issue 6

Sumby P, Whitney AR, Graviss EA, DeLeo FR, and Musser JM. 2006. "Genome-Wide Analysis of Group A Streptococci Reveals a Mutation That Modulates Global Phenotype and Disease Specificity." PLoS Pathog. DOI: 10.1371/journal.ppat.0020005.

Topp S, Reynoso CM, Seeliger JC, Goldlust IS, Desai SK, Murat D, Shen A, Puri AW, Komeili A, Bertozzi CR, Scott JR, and Gallivan JP. 2010. “Synthetic riboswitches that induce gene expression in diverse bacterial species.” Appl. Environ. Microbiol. DOI:10.1128/AEM.01537-10

Treviño J, Perez N, Ramirez-Peña E, Liu Z, Shelburne SA 3rd, Musser JM, and Sumby P. 2009. “CovS simultaneously activates and inhibits the CovR-mediated repression of distinct subsets of group A Streptococcus virulence factor-encoding genes.” Infect Immun. DOI: 10.1128/IAI.01560-08.