THE PENNSYLVANIA STATE UNIVERSITY
THE SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

REGULATION OF σ^E BY ppGpp IN *ESCHERICHIA COLI* AND THE USE OF *fam*P AS A REPORTER FOR SigE IN *BORDETELLA BRONCHISEPTICA*

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SPRING, 2012

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biotechnology
with honors in Biotechnology

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Abstract

When faced with cell envelope stress, the cell employs various sensing mechanisms to activate signal transduction pathways that ultimately lead to changes in its gene expression profile to combat stress. This can be achieved by activating specific transcription factors that allow them to bind to RNA polymerases. One such sigma factor is the alternative sigma factor σ^E. In *E. coli* σ^E is activated upon cell envelope stress such as heat, ethanol stress, and disruptions in the folding of outer membrane porins (1, 3, 4). Upon activation, the anti-sigma factor RseA is cleaved to release σ^E, thus allowing it to bind to RNA polymerase (2, 5). Alternatively, the σ^E pathway can be triggered by ppGpp with the help of the co-factor DksA. ppGpp functions as a global regulator of gene expression during the stringent response. Previous studies have found that regulation of σ^E under phosphate starvation conditions is DksA-independent (9). In this study we found that regulation through ppGpp appears to vary under different starvation stresses. When cells are subjected to carbon starvation, up-regulation of σ^E activity is fully dependent on DksA.

While regulation of σ^E is well characterized in *E. coli*, little is known about the SigE pathway in respiratory pathogen *B. bronchiseptica*. To provide better understanding of the regulation of SigE in *Bordetella sp.*, a reporter has been constructed using the promoter regions of the *fam* gene in *B. bronchiseptica*. SigE is able to transcribe the *fam* promoter in vitro, and the current reporter showed that SigE can similarly transcribe *fam* in vivo in *B. bronchiseptica*. The *fam*P::*lacZ* serves as a tool to shed light on the significance of SigE for the virulence and viability of this respiratory pathogen.

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Introduction

Bacteria are susceptible to the hostile environment that they live in. In order to shelter themselves from external stresses such as changes in osmotic pressure, pH, temperature, etc., they need to be able to sense these changes and subsequently carry out appropriate modifications to increase their fitness. These selective pressures have led to the development of various sensing mechanisms and pathways that together maintain the integrity of the cell envelope. Among these, a pathway mediated by the alternative sigma factor σ^E is the focus of this study.

The outer membrane of Gram-negative bacteria is coated by a thick layer of lipopolysaccharide (LPS). The LPS is involved in all aspects of contact with the external environment, and is essential for viability. It elicits a wide range of immune responses in its host and therefore is termed endotoxin. In pathogenesis, LPS aids in adhesion, virulence and colonization of the host cell. The outer membrane in Gram-negative bacteria contains pore-forming proteins (porins) that allow sugars, ions and amino acids to passively diffuse across the outer membrane (20). The murine lipoprotein located in the periplasmic space tightly links both the outer membrane and the peptidoglycan layer to maintain structural integrity of the bacterial cell envelope (6).

The essentiality of the outer membrane makes preserving its integrity vital. Because countless reactions take place at the surface of the cell envelope, the cell is very effective in sensing environmental stimulus and is fast to react. Commonly, this can be achieved by activating sigma factors and directing them to subsets of specific promoters to alter

gene expression. In *E. coli*, σ^E is an alternative sigma factor encoded by the gene *rpoE* that is activated by stresses that interrupt the integrity of the cell envelope, specifically disruptions in the folding of outer membrane porins (15).

σ^E is essential for viability

Although previously thought to be essential for growth only under high temperatures (16), further investigations revealed that σ^E is needed for viability at all temperatures in *E. coli* (12). Growth of the Δ*rpoE* strains was attributed to suppressor mutations. Growth also ceases when the negative regulator of σ^E, RseA, is overexpressed. σ^E drives transcription of various genes that are needed to maintain cell envelope integrity when bacteria are threatened by various environmental stresses. Thus it is likely that σ^E - dependent genes are involved in keeping bacteria cells viable.

Regulation of σ^E through the RseAB pathway

Under normal growth conditions, σ^E is tethered to the plasma membrane by an anti-sigma factor RseA, which is encoded by the second gene (*rseA*) in the rpoE operon (19). The availability of σ^E is largely dependent on the stability of RseA. *ΔrseA* strains have displayed high levels of σE activity and overexpression of rseA has an inhibitory effect on σ^E activity (19). When subjected to envelope stress, RseA is degraded through the proteases, DegS, RseP, and ClpXP, thus freeing σ^E. σ^E is then allowed to bind to RNA polymerase and direct it to specific promoters (2, 5). A signal transduction cascade to alter gene expression is thus activated upon stress encounter.

The role of Guanosine tetraphosphate (ppGpp) as a starvation signal

ppGpp is a global starvation signal whose levels increase when nutrient levels are low (8,9). Together with its cofactor DksA, ppGpp initiates what is known as the stringent response (7, 10). It binds directly to RNA polymerase to modulate transcription of genes that are required to remodel gene expression. Contrary to the RseAB pathway that regulates σ^E through a series of signal transduction cascade, the ppGpp pathway provides the cell a more direct and energy saving method altering gene expression. Studies in *E. coli* found that regulation of σ^E is dependent upon ppGpp but not DksA during phosphate starvation conditions (9). The situation is σ^E-specific because under similar conditions, DksA is still required to activate σ^S, the master regulator of stress response in *E. coli*. In this study, we attempt to investigate whether DksA is required for the increase in σ^E activity through alarmone ppGpp under carbon starvation conditions. Similar to phosphate starvation, carbon starvation is a slow starvation process. Upon entry to stationary phase, ppGpp levels rise correspondingly. However, little study has been done on its penta-phosphate analogue pppGpp. pppGpp is converted to ppGpp by Guanosine 5'-triphosphate, 3'-diphosphate pyrophosphatase (GppA). Using a GppA mutant, this study has shown that regulation of the stringent response is not specific to ppGpp alone but can be similarly responsive to its analogue pppGpp as well.

σ^E in *E. coli* and B. bronchiseptica

Although the system is generally highly conserved, environmental factors that induce σ^E activity vary across species. In *E. coli*, σ^E is responsible for maintaining cell envelope integrity upon environmental triggers such as heat shock and ethanol stress (18). Its

regulation through the anti-sigma factors RseA and RseB has been studied extensively in *E. coli*. It is induced by the misfolding of outer membrane porins and the subsets of genes induced by σ^E-driven promoters include genes encoding RpoH, cell envelope folding proteins such as FkpA, Skp, SurA (11), as well as 100 other genes. However in *Bordetella*, little is known about its sensing mechanisms. Studies on virulence and adaptation in *Bordetella* sp. mostly revolved around the BvgAS phosphorelay system. However, past studies in the lab demonstrated cell envelope sensing is not limited to BvgAS (22), and the SigE (*Bordetella* σ^E is denoted as SigE in the context of this paper to distinguish from *E. coli* σ^E) pathway has also proved to be significantly important in modulating gene expression (Barchinger, results unpublished).

The fam promoter region

The *fam* gene, also known as *rpoH*, encodes for an alternative sigma factor (σ^{32}) that is mainly responsible for induction of expression of heat shock related genes. Transcription of the fam gene is driven by several sigma factors, including σ^{70} and σ^E-driven promoters. For that reason, we attempted to create a reporter construct using the promoter region of *fam*. The rationale behind creating such a construct is to monitor σ^E activity in *Bordetella* cells. In the current work, two reporter constructs using varying lengths of the promoter regions of *fam* have been created to test their relative responsiveness to predicted σ^E activity in *B. bronchiseptica* cells.

Chapter 1

Materials and Methods

Beta-galactosidase Assays.

Cultures were grown overnight at 30˚C in a roller drum with aeration and subcultured to an OD_{600} of 0.025 the following day. Subcultures were grown in 20 ml of EZ-rich media at 30˚C in 125 ml flasks with shaking in a gyratory water bath. Throughout the growth curve, 0.5ml of sample was taken at intervals of 30-40 minutes for the assay and added to respective assay tubes containing 0.5 ml Z buffer, 1.4 µl of beta-mercaptoethanol, one drop of 0.1% SDS, and 2 drops of chloroform. OD_{600} of cultures were measured at the time of each sampling. Samples were collected until culture reached stationary phase, in which minimal further increase in OD was observed.

Prior to the assay, beta-galactosidase samples were incubated at 28˚C for 10 minutes.
200 µl of ortho-Nitrophenyl-ß-galactoside (ONPG) at a concentration of 4 mg/ml were added to initiate the reaction. The reaction was terminated with 0.5 ml 1M Na_2CO_3 when a color change from transparent to bright yellow was observed. Subsequently, samples were centrifuged for 5 minutes to remove cell debris and the OD_{420} of supernatant were measured.

All strains used contained the *rpoHp3-lacZ* reporter construct. Thus, σ^E activity can be observed through beta-galactosidase activity assays. σ^E activity is monitored in constant intervals throughout the entire growth period and determined by plotting a differential rate plot (beta-galactosidase activity/0.5 ml cells vs. OD_{600}).

Media

In experiments investigating the role of DksA in increasing σ^E activity under carbon starvation, MOPS defined media without amino acids were used. The media contained 10X MOPS buffer, 10X ACGU mix, 0.132 M K_2HPO_4 in distilled water. 5X EZ supplement was excluded to deprive the media of amino acids, another source of carbon. High (0.2%) and low (0.02%) concentrations of glucose were used as carbon source in these experiments. EZ-Rich media (low phosphate) containing 0.02% phosphate (as opposed to 0.2%) was used in the experiment testing the ability of Δ*gppA* mutant in regulating σ^E activity.

Strains and Growth Conditions

A complete list of strains used in this study can be found in Table 1. All *E. coli* strains in this set of experiments were cultured in a 30˚C gyratory water bath with aeration. Overnight cultures were incubated in rotating roller drum at 30˚C for 14-16 hours.

Table 1. Complete bacterial strains and plasmids

	Strain	Genotype	Resistance	Strain Description
E. coli	SEA001	MG1655 Φλ*rpoH*P3::*lacZ* ΔlacX74		
	SEA6020	MG1655 Φλ*rpoH*P3::*lacZ* ΔlacX74	Tet^R	Δ*dksA*
	SEA7194	MG1655 Φλ*rpoH*P3::*lacZ* ΔlacX74	Kan^R	Δ*gppa*
	SEA008	SEA001 pTrc99a	Amp^R	
	XQZ004	DH5α pXQZ004	Tet^R	
	DH10B	DH10B	Tet^R	
B. bronchiseptica	RB50	RB50	$Strep^R$	
	SEA5516	RB50Δ*sigE*	Tet^R, $Strep^R$	
	SEA5517	RB50Δ*rseAB*	Tet^R, $Strep^R$	
	SEA5543	RB50 pMP220	Tet^R, $Strep^R$	
	SEA5544	RB50Δ*sigE* pMP220	Tet^R, $Strep^R$	
	SEA5545	RB50Δ*rseAB* pMP220	Tet^R, $Strep^R$	
	SEA5540	RB50 pFam (short)	Tet^R, $Strep^R$	
	SEA5541	RB50Δ*sigE* pFam (short)	Tet^R, $Strep^R$	
	SEA 5542	RB50Δ*rseAB* pFam (short)	Tet^R, $Strep^R$	
	SEA5547	RB50 pFam (short)	Tet^R, $Strep^R$	
	SEA5550	RB50Δ*sigE* pFam (short)	Tet^R, $Strep^R$	
	SEA 5552	RB50Δ*rseAB* pFam (short)	Tet^R, $Strep^R$	

Results

Activation of σ^E is not DksA-dependent under carbon starvation conditions

Figures 1 and 2 shows the result of the experiment in which σ^E activity in a Δ*dksA* strain is compared with that of the wild type. It was found that under normal stationary phase conditions, σ^E activity is DksA independent. Both wild type and Δ*dksA* strains had comparable levels of activity as seen in the differential rate plot. When the strains were grown under carbon starvation conditions, similar results were observed. Cells entered stationary phase prematurely at an OD_{600} of 0.35, and σ^E activity levels were comparable in both wild type and Δ*dksA* strains.

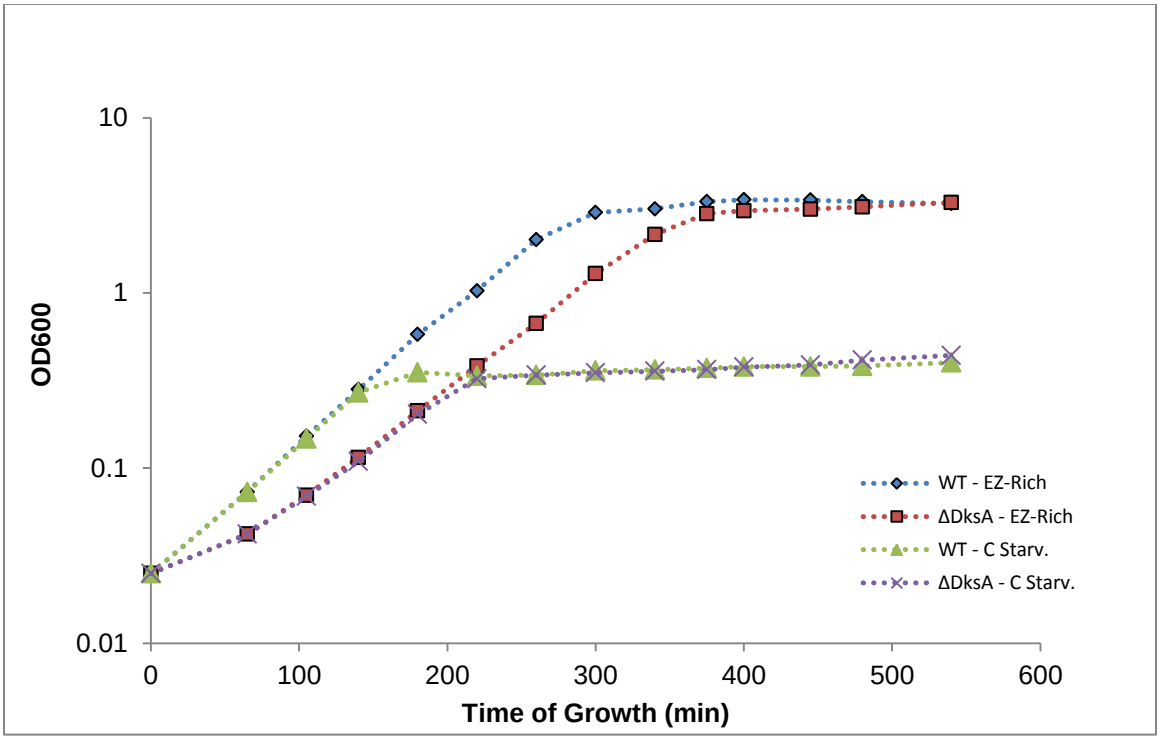
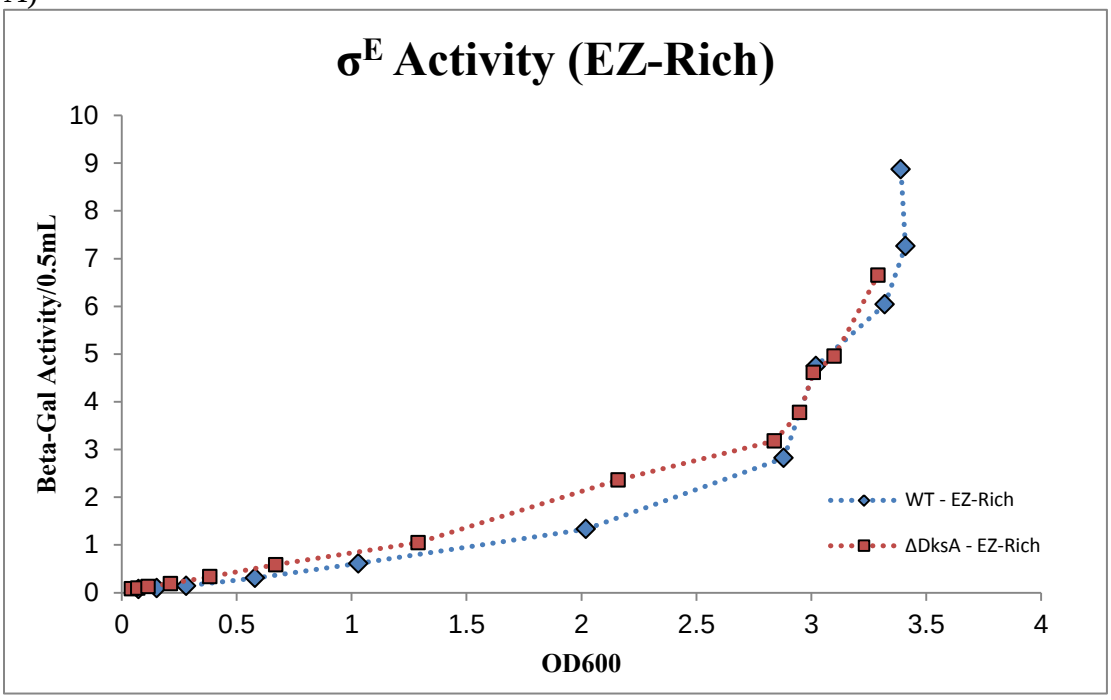


Figure 1. Growth curves of wild type (SEA001) and Δ*dksA E. coli* strains under MOPS defined media lacking amino acids.

A)



B)

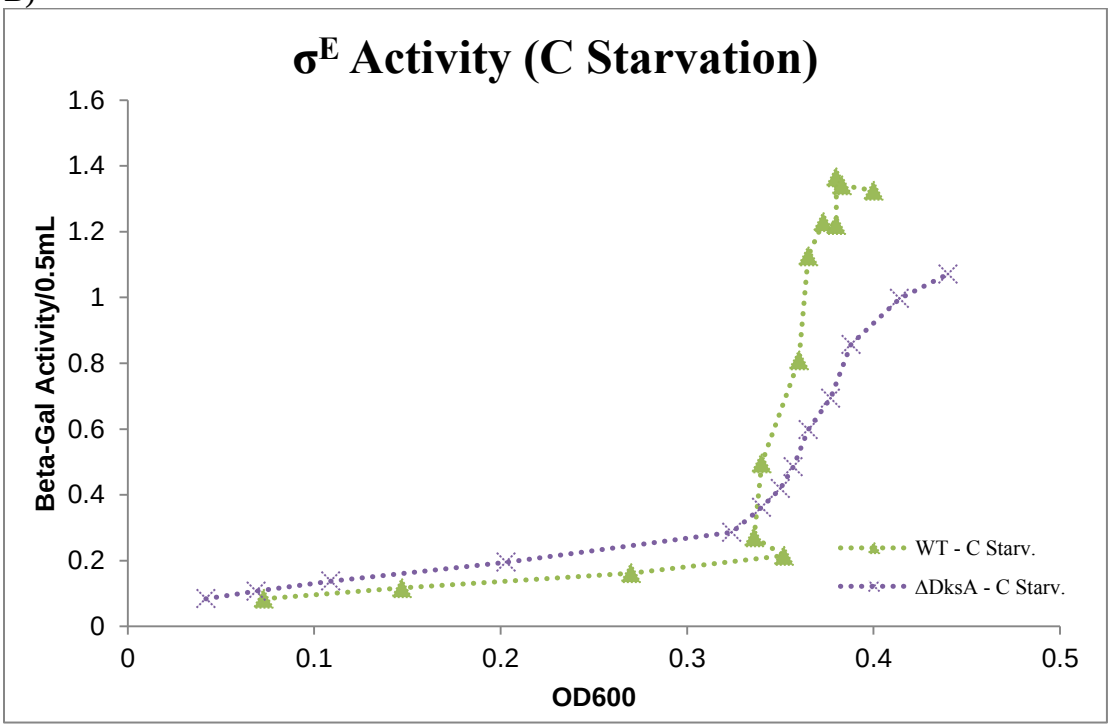


Figure 2. σ^E activity as measured by ß-galactosidase assay on wild type (SEA001) and Δ*dksA E. coli* strains in (A) EZ-Rich media and (B) MOPS defined media without amino acids. Changes in the slope observed between any two points represent changes in σ^E activity in that period. Thus synthesis of new ß-galactosidase at any given period can be directly observed through the differential rate plot.

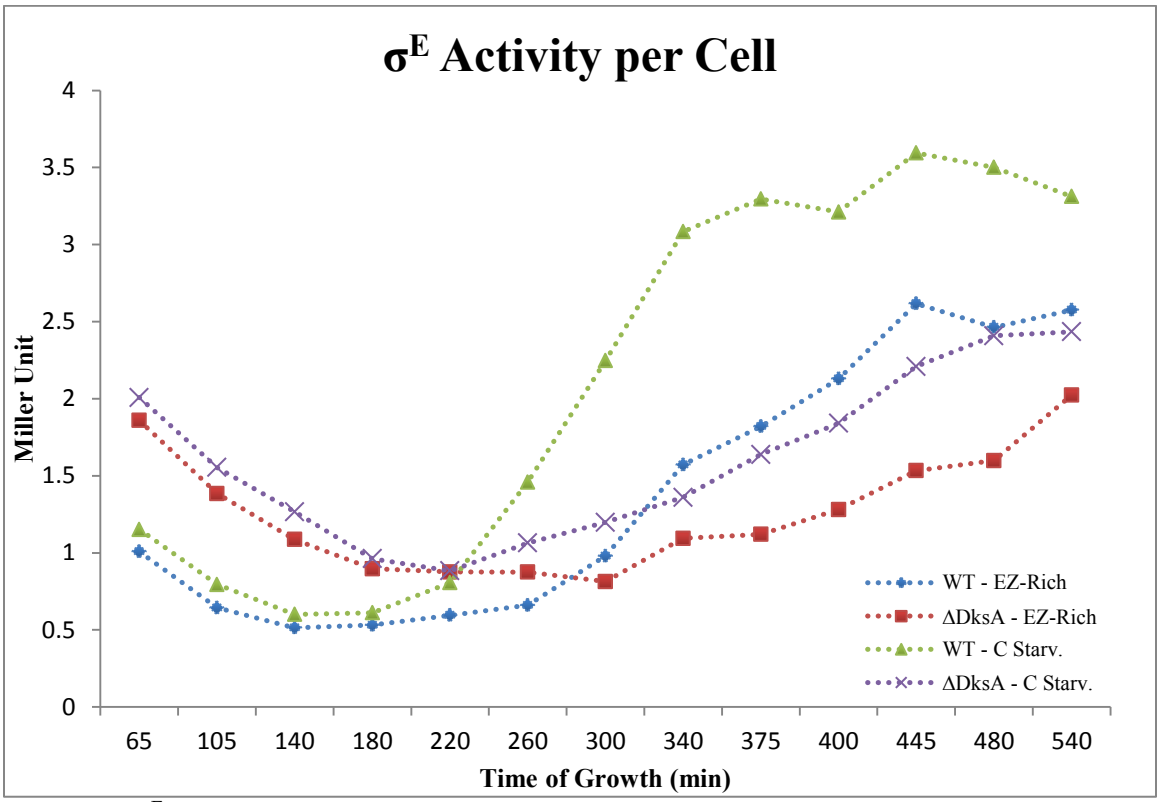


Figure 3. σ^E activity is normalized by dividing activity per 0.5 ml with OD_{600} to obtain activity per cell, denoted as Miller Units.

σ^E activity can be regulated by either ppGpp or pppGpp under phosphate starvation

In the *ΔgppA* strain (Figures 4 and 5), σ^E activity was observed to be similar to that of the wild type strain. σ^E activity increases as cells enter stationary phase. When cells were subjected to phosphate starvation, it can be seen from the growth curve that cells entered stationary phase earlier, and σ^E activity increased in a corresponding manner in both the wild type and *Δgpp*A strains.

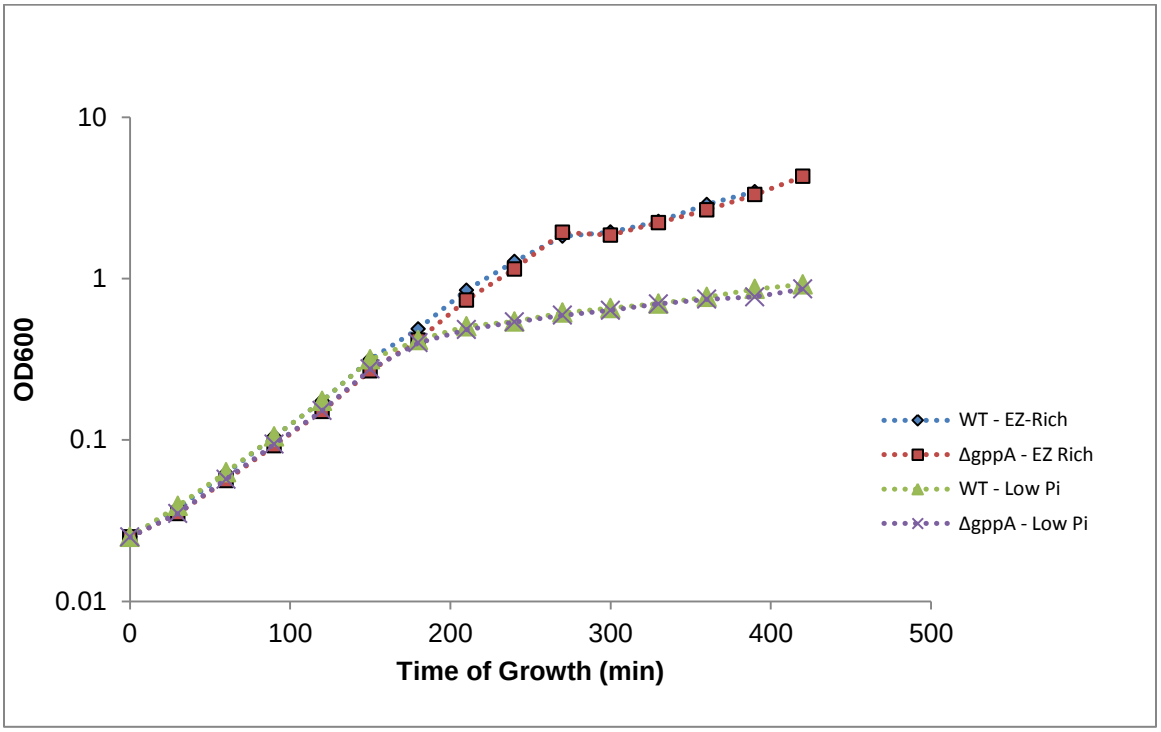
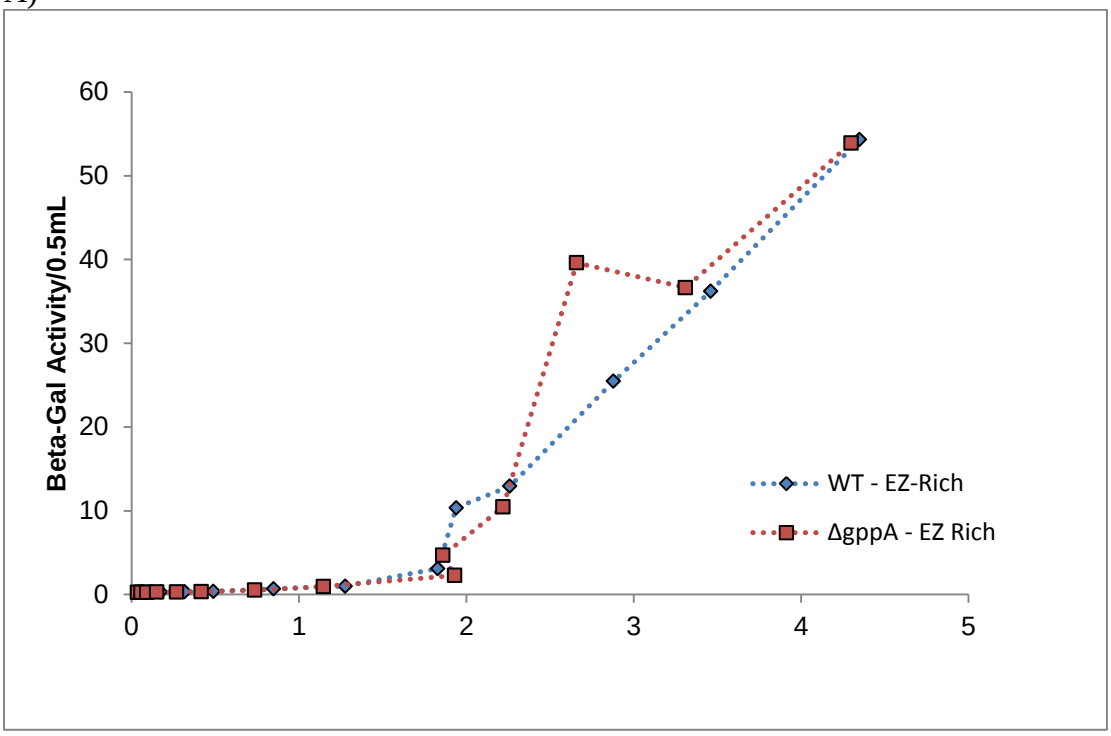


Figure 4. Growth curve of wild type (SEA001) and Δ*gppA E. coli* strains grown in EZ-Rich media and EZ-Rich 0.02% phosphate (low phosphate).

A)



B)

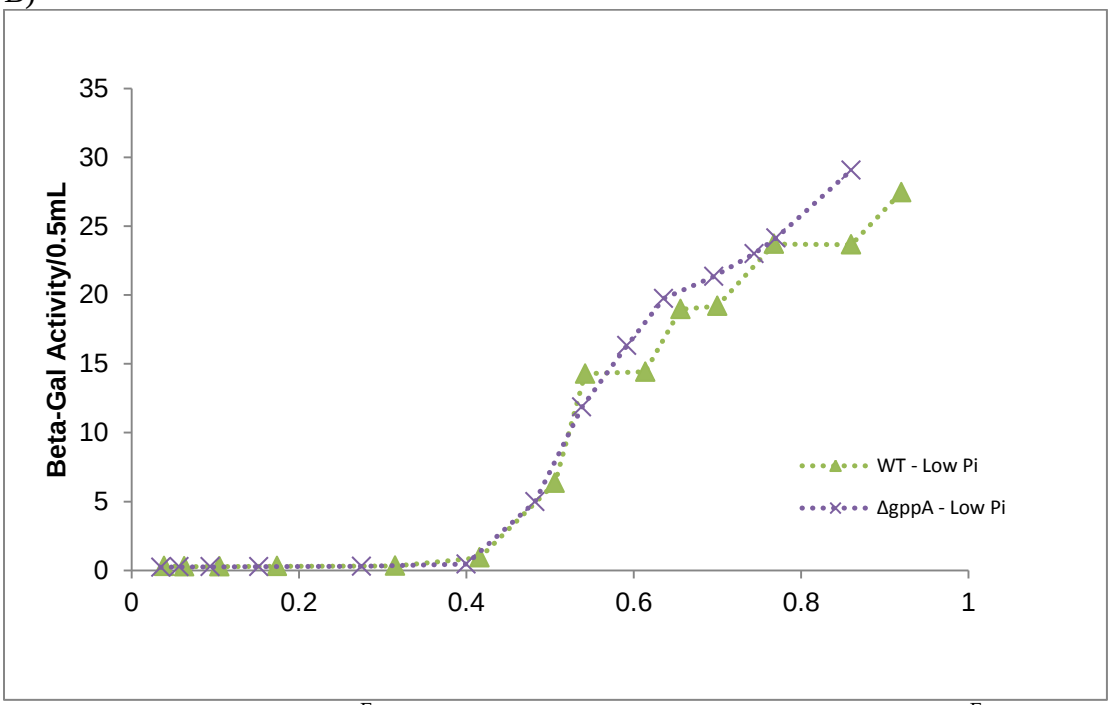


Figure 5. Corresponding σ^E activity of the growth curve in Figure 3. σ^E activity was measured through ß-galactosidase assay on wild type (SEA001) and Δ*gppA E. coli* strains grown in (A) EZ-Rich media and (B) EZ-Rich with reduced phosphate concentration (0.02%).

Discussion

The regulation of alternative sigma factor σ^E largely involves negative regulation by RseA, an anti-sigma factor that will be degraded to release σ^E when there are disruptions in folding of outer membrane porins (2, 5, 15). It has also been discovered that σ^E activity increases as cells enters stationary phase, when nutrient levels decrease (7). This increase in σ^E activity is caused by elevated levels of ppGpp, an alarmone that serves as a global signal for starvation-induced stress (Cashel *et al*., 1996). In environments where nutrients are scarce, cells produce higher levels of ppGpp and this in turn, resulted in higher transcriptional levels of σ^E. This regulation pathway is dependent on ppGpp, which contrasts with the RseA pathway mentioned earlier.

ppGpp and its cofactor DksA bind directly to RNA polymerase to regulate global transcription of several sigma factors, including the housekeeping sigma factor σ^{70} in response to nutrient availability. It has been shown that the activity of σ^E in *E. coli* increased proportionately when cells were subjected to phosphate starvation (9), but the effects were independent of DksA. We set out to investigate if the same result prevails when carbon is the limiting nutrient. Results showed this is indeed the case. Both in the wild type and the Δ*dksA* strain, σ^E activity continued to increase as cells proceed into carbon starvation phase. In contrast with EZ-rich medium with 0.2% glucose, cultures enter stationary phase at OD0.35 with the modified MOPs medium with 0.02 glucose. Although the general trend in increase of σ^E activity follows, overall activity is much lower due to the fact that cells were not able to synthesize as many beta-galactosidase enzymes in carbon starvation state. As samples were collected at each time point, cells

remaining in the flasks remains constant, thus σ^E activity can be normalized by dividing activity per 0.5 ml cells with OD_{600} to obtain activity per cell, denoted as Miller Units. As seen in Figure 3, σ^E activity per cell in the Δ*dksA* and wild type strains did not increase significantly when cells are subjected to starvation.

pppGpp as a Substitution for ppGpp in the regulation of σ^E activity

To investigate if the penta-phosphate form (pppGpp) of ppGpp works in a similar fashion as its tetra-phosphate analogue, a Δ*gppA* mutant was subjected to growth under phosphate starvation. GppA serves as a phosphatase to dephosphorylate pppGpp into its tetra-phosphate form of ppGpp. The reaction cannot proceed in the absence of GppA, thus only the penta-phosphate form is present in our pool of alarmones. We observed that under this condition, σ^E activity was upregulated to the same extent even in the absence of ppGpp. The differential rate plot obtained from the ΔGppA mutant continues with an upward-sloping trend until stationary phase, indicating that σ^E activity regulation by ppGpp is not specific to its tetra-phosphate analogue. In other words, pppGpp can regulate σ^E as well.

Chapter 2

Materials and Methods

Strains and Media

A complete list of strains in this study can be found in Table 1. *E. coli* with a chromosomal *rpoH*P3::*lacZ* fusion was grown on LB at 37 °C, with 100 μg/ml ampicillin or 20 μg/ml tetracycline, when appropriate. *Bordetella bronchiseptica* strains were plated on Bordet-Gengou agar (Difco) with 10% defibrinated sheep blood (Hema Resources). In liquid culture, *B. bronchiseptica* was grown in Stainer-Scholte broth (Stainer). 20 μg/ml streptomycin and 20 μg/ml tetracycline was added to each media as necessary.

PCR of *fam* promoter region from *B. bronchiseptica*

The *fam* promoter region was PCR-amplified from genomic DNA of *Bordetella bronchiseptica* as follows: polymerase chain reaction (PCR) was performed using Taq polymerase using the Peltier Thermal Cycler. The 50 μl reaction mix was made up of 1 μl of *B. bronchiseptica* genomic DNA, 1 μl of forward primer, 1 μl of reverse primer, 1μl of deoxynucleotide phosphates (dNTP), 0.5 μl Taq polymerase, 5 μl 10X reaction buffer and 40.5 μl dH_2O.

Table 2. Primers for short and long famP amplification

Primer	Sequence
*fam*P long (forward)	GCCGCCGAATTCATAAGGGACGCATAGAA
*fam*P long (reverse)	GCGGCGCTGCAGGGAACGATGTTAGCACTC
*fam*P short (forward)	GGGCGGGAATTCTGCCGTTCGTGGATGTCCAG
*fam*P short (reverse)	GGGCGGAAGCTTGGGCCAACGAACTACTGGGT

Plasmid construction of short and long reporters *fam*P*::lacZ*

The plasmid pMP220 was used as an expression vector for cloning the promoter *fam*P. pMP220 contains a promoterless *lacZ* and a gene for tetracycline resistance. Restriction digestions of the *fam* PCR product and pMP220 were performed with restriction enzymes EcoR1 and Pst1. Both utilized a reaction mixture of 5 µl of *fam*/pMP220, 0.3 µl BSA, 1.5µ lof EcoR1, 1.5 µl of Pst1, 3 µl of 10X reaction buffer, brought to a total of 30 µl with distilled H_2O, and incubated at 37 °C for at least one hour, then the appropriate fragments were separated on an agarose gel and isolated by gel extraction (Qiagen). Digested fragments of both the PCR fragment (*fam* promoter region) and the cloning vector (pMP220) were ligated using T4 DNA ligase (NEB). The ligation mixture contained 2 µl of cut insert, 2 µl of cut vector, 0.5 µl ligase, 1.5 µl 10X T4 DNA ligase, and brought to a total of 15 µl with distilled H_2O. For cloning the short *fam*P construct, pTrc99a expression plasmid was used instead of pMP220.

Transformation of *fam*P*::lacZ* into *E. coli*

Ligations were subsequently transformed into competent cells of *E. coli* strain DH10B and plated onto LB plates containing tetracycline. Uptake of DNA was done through heat shock. Cells were incubated on ice for 25 minutes prior to the heat shock process and subjected to 1 minute of incubation in a 42 ˚C water bath. The cells were then iced for 5 minutes. Sterile LB media was added and cells were allowed to grow in 30˚C for 1 hour. 100 µl of culture were plated on LB plates containing 20 µg/ml tetracycline for selection. Plates were incubated for 12-16 hours at 37 ˚C.

*fam*P*::lacZ* uptake by *Bordetella*

The reporter construct *fam*P*::lacZ* was mobilized from *E. coli* to *Bordetella bronchiseptica* by tri-parental mating as follows: wild type *B. brochiseptica* strain RB50, *E. coli* helper strain containing the *tra* gene to facilitate transfer of DNA, and *E. coli* strain DH5α harboring reporter *fam*P*::lacZ* were mated into a single colony and struck on Stainer-Scholte plates containing 10 mM $MgCl_2$ without antibiotics and incubated at 37˚C for 3 hours prior to being re-struck. The colony was subsequently plated on Bordet-Gengou (BG) agar containing 20 μg/ml tetracyline to select for *Bordetella* strains that took up the plasmid, and 20 μg/ml streptomycin to select against *E. coli*.

Beta-galactosidase Assays

To test the responsiveness of our putative reporters, both short and long *fam*P constructs were moved into *B. bronchiseptica* wild type strain (RB50), RB50Δ*sigE* and RB50Δ*rseAB* strains. Duplicate cultures started from dilutions of a single colony resuspended in Stainer-Scholte broth were grown overnight in 37 ˚C roller drum with aeration. The OD_{600} of each culture was measured, and duplicate samples of 0.1 ml were added to 0.9 ml of Z-buffer, 1.4 μl of beta-mercaptoethanol, one drop of 0.1% SDS, and 2 drops of chloroform. Prior to the assay, samples were incubated at 28˚C for 10 minutes. 200μl of ortho-Nitrophenyl-ß-galactoside (ONPG) at a concentration of 4 mg/ml were added to initiate the reaction. The reaction was terminated with 0.5 ml 1M Na_2CO_3 when a color change from transparent to bright yellow was observed. Cell debris was spun out, the yellow color intensity was quantified at OD_{420} and Miller units were calculated ($1000*[OD_{420}/time]/OD600$).

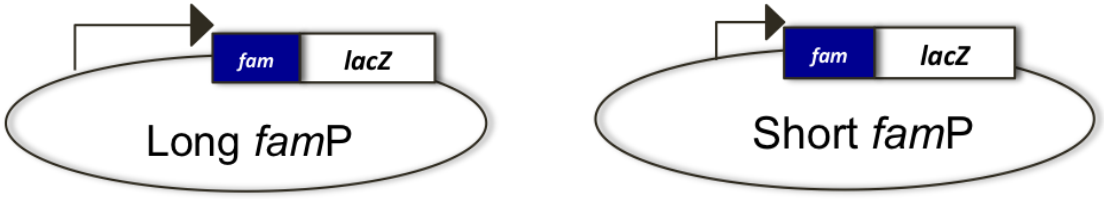
Results

Construction of a SigE reporter in *B. bronchiseptica*

SigE of *Bordetella* is similarly encoded by *rpoE* as in *E. coli*. As previously described, σ^E in *E. coli* recognizes the promoter of *rpoH*P3 and subsequently a *rpoH*P3::*lacZ* fusion was created to monitor σ^E activity in *E. coli* cells. While experiments have been done using *Bordetella* SigE in directing transcription of *E. coli rpoH*, the question of whether SigE can recognize and direct transcription of its own *rpoH* (*fam*) in vivo remains unclear. Thus it is important that we create a reporter using the *fam* promoter region of *B. bronchiseptica* to test its responsiveness in SigE-dependent transcription in *Bordetella sp*. We successfully fused two *Bordetella fam* promoters varying in length with *lac*Z. When SigE is released to bind to *fam*P, downstream genes that follow, including *lacZ*, will be transcribed. Thus SigE activity corresponds to *lacZ* activity and can be directly inferred from measuring amounts of ß-galactosidase produced by *lacZ* through a ß-galactosidase assay.

Results obtained from the gel containing amplified long *fam*P construct (including approximately 200 bp upstream of the beginning of the *fam* open reading frame) indicates that the plasmid containing reporter *fam*P*::lacZ* was successfully taken up by *E. coli* strain DH10B. Twelve single colonies were selected and PCR-amplified for their reporter plasmid using primers pMP220 upstream sequences and pMP220 downstream sequences for validation purposes (Figure 5). Bands on lanes 1-12 showed amplified long *fam*P, whereas lane 13 is *fam* obtained from *B. bronchiseptica* genomic DNA, without the the reporter-carrying plasmid pMP220. Upon successful cloning, the construct was moved

into the streptomycin-sensitive strain DH5α, and tri-parental mating was performed to move the plasmid from DH5α to the indicated *B. bronchiseptica* strains, and small, isolated colonies were observed on selective BG plates containing 20 µg/ml tetracycline and 20 µg/ml streptomycin. Subsequently, short *fam*P (including only the predicted SigE-dependent promoter) was cloned into *B. bronchiseptica* in a similar fashion (Barchinger, data unpublished).



ATAAGGGACGCATAGAAAAATATCCTGCGT ACTGGGAAATTCCGGAACTTTGGCGCGGCG CAGCAGTCGTATTAGCACTCGTTGCGTGGA GTGCTAACATCGTTCC

CGGAACTTTGGCGCGGCGCAGCAGTCGTA

Figure 6. Reporter constructs long/short *fam*P and respective promoter regions. The -35 and -10 promoter regions, which are recognized by SigE, are shown in red.

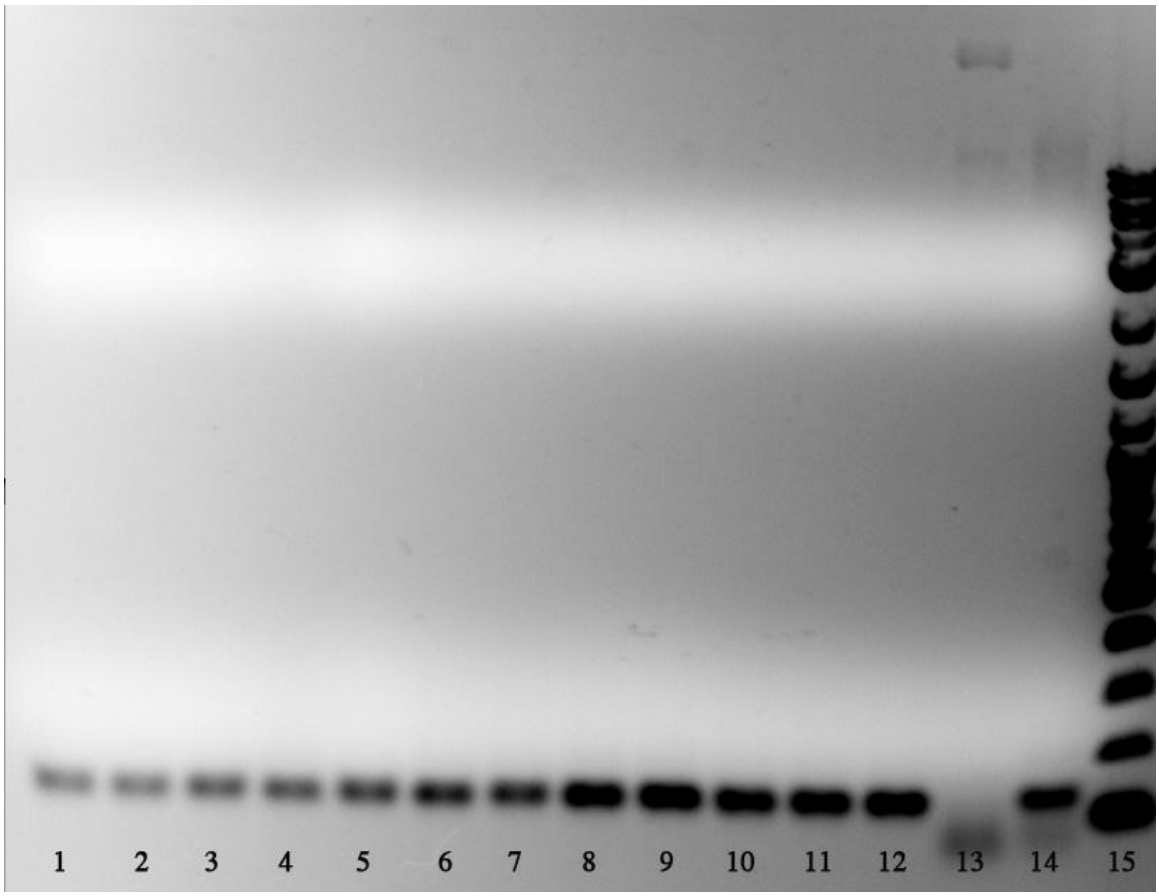


Figure 7. Check gel (1.2% agarose) on amplified long *fam*P extracted from *E. coli* DH10B. Lanes 1-2: long famP; lane 13: *B. bronchiseptica fam* gene. Lane 14: MW markers. Label the lanes on the gel. Show the size of the DNA markers

Short *fam*P is more responsive than *fam*P in measuring σ^E activity

The cloned reporters short *fam*P and long *fam*P both demonstrated responsiveness to σ^E activity (fig.). The short *fam*P reporter (with a shorter upstream promoter sequence) is more responsive to SigE-dependent transcription. As seen in Figure 8, RB50Δ*sigE* strains using both the short and long *fam*P displayed residual levels of SigE activity, and conversely RB50Δ*rseAB* (lacks negative regulators of SigE activity) had high levels of SigE activity, indicating that our reporter is responsive to SigE-dependent transcription of

lacZ using *fam*P. The two different reporters with varying lengths in their promoter regions differed in responsiveness. In the same set of experiments, reported SigE activity in RB50ΔRseAB using short *fam*P was more than 10 Miller units higher that of the long promoter. It can also be seen from the results that short *fam*P reported a larger difference in SigE activity between RB50 and RB50Δ*sigE*, which the long *fam*P failed to detect.

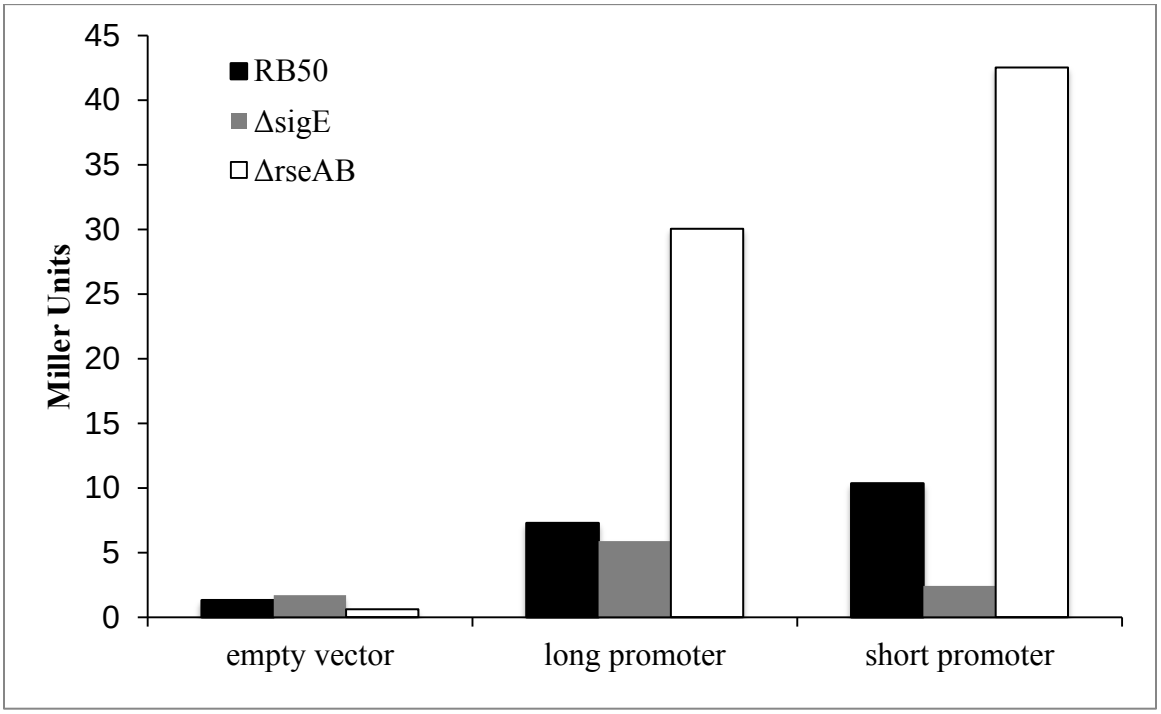


Figure 8. Miller plot on SigE activity in strains RB50, RB50Δ*sigE*, and RB50*rseAB*. Reported SigE activity is compared between the long and short *fam*P reporters.

Discussion

σ^E in *E. coli* is encoded by the gene *rpoE*. Promoter regions of *rpoH*, the gene encoding another sigma factor that controls the heat shock response, can be recognized and transcribed by σ^E. In the previous set of experiments involving regulation of σ^E by ppGpp (Chapter 1), the *rpoH*P3*::lacZ* reporter was utilized to observe *sigE*-dependent transcription. Because *rpoH*P3 is specific to *E. coli*, the same cannot be applied in our *Bordetella* system. While *B. bronchiseptica* SigE does recognize and direct transcription of the *E. coli rpoH*P3 promoter, it had not yet been determined whether *B. bronchiseptica* SigE does, in fact, direct transcription of its own *rpoH*(*fam*) gene in vivo. In this work, we showed that *B. bronchiseptica* SigE recognizes the promoter regions of *rpoH* (also known as *fam* in the current context). *rpoH*P3 (*E. coli*) and *fam* (*B. bronchiseptica*) possess similar, but not identical sequences at the -35 and -10 promoter regions, but the spacer varies across species. Thus, the rationale behind building reporter *fam*P*::lacZ* unique to *Bordetella* is to investigate whether the basic regulatory module of σ^E by RseAB as seen in *E. coli* is conserved in *Bordetella*.

To test the activity of the long *fam*P construct, we found that *lacZ* activity did not significantly decrease in the RB50Δ*sigE* strain. In addition to the *fam* promoter sequences, the long *fam*P includes approximately 200 bp upstream of the beginning of the *fam* open reading frame. Thus it might be possible that those extra regions in upstream contained other regulatory promoters that are not SigE-dependent. It is important then, for us to omit unnecessary noise by constructing a reporter to obtain results that are more indicative of σ^E-dependent transcription. From Figure 8, it can be observed that short

*fam*P fulfilled this role. Short *fam*P reported higher levels of SigE activity in RB50ΔrseAB and lower levels in RB50ΔsigE compared to long *fam*P, indicating that specificity towards σ^E-dependent transcription increased. This is based on the assumption that the short *fam* promoter in RB50 is recognized uniquely by SigE.

From the same set of results we also showed that regulation of σ^E in *E. coli* by repressor proteins RseA and RseB is conserved in *Bordetella sp*. SigE activity increased for both the short and long constructs in the strain lacking rseA and rseB. Using the current reporter, further testing can now be performed to investigate the different factors that induce σ^E activity in *Bordetella*. σ^E has been found to play different roles in different bacteria. By gaining more insights into how the system is regulated in respiratory pathogens such as the *Bordetella* species, we are a step closer to discovering a suitable inhibitor that would block these stress-sensing mechanisms, therefore defeating the rise of drug resistant pathogens.

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Work Experience

Biologicals Research Intern *May – August 2011*
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- Investigated the effects of synthetic cationic peptides on the production of extracellular proteins in different bacteria through amino acid analysis
- Devised protocols for the fractionation and isolation of *B. subtilis* cytosolic proteins
- Obtained proficiency in working with S2 level organisms and radioactivity
- Analyzed and presented current literature on the issue of antibiotic resistance in the United States

Pharmaceutical Products Division Intern *May - August 2010*
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- Lead other interns in referencing and information proof read in the production of annual detail advertisement posters
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Research Experience

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- Investigated cellular roles of transcription factor Sigma (E) of *E. coli* in regulating signaling pathways to maintain bacterial cell integrity under environmental stress
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