Exploring the functional role of the bacterial protein SeqA and co-occurring genome stability systems in TnsE-mediated Tn7 transposition using the *E. coli* model system

Honors Thesis

Presented to the College of Arts and Sciences,

Cornell University

in Partial Fulfillment of the Requirements for the
Biological Sciences Honors Program

by

Alan Chramiec

May 2013

Supervisor Joseph E. Peters, Ph. D.

Abstract

Transposon Tn7, is unique among transposons for both its target specificity and selectivity. One of its target selection pathways, mediated by the protein TnsE, has been found to allow Tn7 to preferentially transpose into mobile plasmids despite there only composing approximately 1% of the bacterial genome. Prior research in the Peters laboratory revealed both a physical interaction between TnsE and the bacterial protein SeqA as well as a functional role of the TnsE-SeqA interaction in TnsE-mediated transposition. Based on this work a model was developed, whereby SeqA occludes TnsE's access to the replicating chromosome. An unrelated study from another group found that the genes *yfbV, metJ, holD, mutH,* and *matP* co-occurred with *seqA* in genomes with an ancestral *dam* gene. In this project, an *in vivo* Tn7 transposition assay was used to investigate the role of the SeqA-TnsE interaction in the TnsE pathway of Tn7 transposition. The same transposition assay was also used on the co-occuring set of bacterial proteins in order to identify a possible association with their gene products and TnsE-mediated transposition. The Tn7 transposition events were then mapped using two complementary assays. The results suggest that in the absence of SeqA, TnsE-mediated Tn7 transposition increases significantly and that a much higher percentage of the insertions occur into the chromosome in a Δ*seqA* background, thus supporting our model. The results for the other proteins were much less clear, with the only significant change in TnsE-mediated transposition frequency occurring in the *ΔmetJ* mutant, where no successful Tn7 transpositions were observed within the detection of our assay.

Introduction

Transposons, first discovered by Barbara McClintock, are DNA elements that are capable of moving between DNAs within the cell (Jones 2005). While there exist a variety of types of DNA transposons, the one studied extensively in this project was Tn7. It relies on five different proteins (TnsA, TnsB, TnsC, TnsD, and TnsE) for transposition, which can occur via two different pathways (Waddell 1988). It is a transposon of incredible interest because it demonstrates both target specificity and selectivity, and because of its presence across a broad variety of bacterial families in very different environments (Li 2013).

Normal transposition by Tn7 involves a cut-and-paste mechanism, where the TnsA subunit of its transposase makes nicks at the 5' ends of the Tn7 element and the target DNA, while the TnsB subunit of the transposase makes breaks at the 3' ends (Li 2013). In the presence of the target, the Tn7 element gets hydrolyzed and TnsB joins the 3' OH ends to target DNA, with repair occurring at the remaining 3' OH ends with DNA polymerase I from the host. TnsC acts as the regulator of this TnsAB transposase by interacting with the target selection proteins TnsD or TnsE in the resulting complex at the target site (Peters 2001).

The focus of this project was the TnsE-mediated Tn7 transposition pathway. TnsE appears to target discontinuous DNA replication and as a result readily transposes into conjugal plasmids as they enter a new host, since they replicate in a discontinuous process during entry (Wilkins and Lanka 1993 and Wolkow et al., 1996). It is this target selection of conjugal plasmids that allows for horizontal

dissemination of Tn7 and could, in concert with the TnsD-mediated pathway's target selection for a "safe haven" in the bacterial chromosome, explain its wide-spread distribution across varying bacterial families (Li 2012). Although at a much lower frequency, the TnsABC+E machinery also preferentially targets the DNA replication terminus as well as double strand breaks (Peters and Craig, 2000 and Shi et al., 2008). In particular, it is believed that TnsE binds to the β clamp processivity factor of DNA Polymerase III holoenzyme, thus explaining why it preferentially targets DNA structures with free 3' recessed ends during lagging strand synthesis (Parks 2009). Prior research has supported the idea that there is a weak interaction between TnsE and the β clamp (Parks 2009). Furthermore, research suggests that in a TnsE-mediated system where DNA gap substrates were populated with β clamps, Tn7 transposes in only one orientation at the 3'-ends of the single strand gaps (Parks 2009).

These studies of TnsE in the Peters laboratory led to further research into the cellular mechanisms involved in the regulation/control of TnsE-mediated Tn7 transposition. Because transposons are generally viewed as parasitic due to their tendency to cause a variety of deleterious mutations, it would be advantageous for the bacterial cell to evolve mechanisms that inhibit this and other alterations that can occur to the genome. A genetic screen revealed that the inactivation of the *dam* gene (coding for Dam methylase) stimulated TnsE-mediated transposition (Li 2012). Previously, Dam had been shown to play a role in the regulation of Tn5 and Tn10 transposon activity by its methylation of GATC sites in either the promoters of the respective transposases or in the binding sites of the recipient DNA elements

(Roberts 1985 and Yin 1988). Further studies however, revealed that the hemi-methylation of the GATC site in the Tn7, performed by Dam methylase, itself did not suppress the stimulation in TnsE-mediated Tn7 transposition seen in *dam* mutants (Li 2012).

The increase in the frequency of TnsE-mediated transposition identified in *dam* strains led to an investigation of the bacterial replication protein SeqA's interaction with TnsE. SeqA has a variety of roles during bacterial chromosome replication. Firstly, it acts as a negative modulator of initiation of replication as it prevents premature reinitiation of chromosome replication by one-third of a generation time by sequestering the replicated origin (Lu 1994). This is largely achieved by the regulatory role of SeqA in slowing the rate of methylation by Dam of the replication origin's hemimethylated GATC sites. The alteration of the rate of methylation of these sites consequently alters the ability of DnaA to initiate chromosomal replication. Secondly, and perhaps most pertinently to this study, SeqA has been found to bind throughout the chromosome to hemimethylated GATC sites that arise after the passage of the replication fork (Brendler et al 2000, Onogi et al 1999).

Initial work pointing to a possible role of SeqA in Tn7 transposition involved an affinity purification-mass spectrometry strategy that found an interaction between a variety of bacterial proteins, one of which was SeqA, with TnsE (Li 2012). The strategy involved running *E. coli* cell lysates through an affinity column with TnsE protein that was linked to the column matrix via a six-Histidine tag, eluting off

proteins that had bound to the TnsE in the column, isolating them using SDS-PAGE, and finally identifying them using mass spectrometry (Li 2012). Single gene knockout strains were then made for many of the proteins identified by mass spectrometry and were tested for any effects on TnsE-mediated Tn7 transposition frequencies via an *in-vivo* transposition assay: *seqA* was found to cause significant changes in transposition frequency. The physical interaction between SeqA and TnsE was further studied using a protease-footprinting assay (Li 2012). It was found that SeqA could retard trypsin from cleaving TnsE at multiple trypsin sensitive sites, strongly supporting a physical interaction between the two.

The functional role of the TnsE-SeqA interaction *in vivo* was then investigated via a lambda hop assay (see Materials and Methods). The results of the assay found a ten-fold increase in TnsE-mediated Tn7 transposition in a *seqA* mutant compared to a wild-type background (Li 2012). Moreover, it was found that the *seqA* knockout only had this effect on TnsE-mediated transposition, as the lambda hop in a system with a mutated TnsABC core machinery (TnsABC*) failed to show a similar increase in Tn7 frequency. The mutant core machinery served as a control that allowed for the observation of effects specific to TnsE target selection because it featured a gain of activity mutation in the TnsC (TnsC^{A225V} or TnsC*) protein that allowed the TnsABC* machinery to promote Tn7 transposition without either the TnsE or TnsD proteins. In the wild type setting TnsABC will not allow any transposition without the TnsD or TnsE proteins and the targets that they recognize. Because *seqA* has a variety of pleiotropic phenotypes in the cell it was unclear why *seqA* inactivation was leading to an increase in TnsE-mediated transposition.

Further genetic studies revealed that DNA replication overinitiation, DNA replication asynchrony, and likely changes in global gene expression—all phenotypes of *seqA* inactivation, were not responsible for the stimulatory effect on transposition (Li 2012).

Similar studies revealed though that another phenotype of *seqA*, the organization of sister chromosomes following replication, likely had an effect on transposition. Based on these studies a model was proposed for the role of the SeqA in TnsE-mediated Tn7 transposition. Following DNA replication, SeqA organizes the replicated chromosomes by binding to the hemimethylated GATC sites that have become temporarily available, and may form a complex between the nascent DNA strands replicated as leading- and lagging-strand templates (Li 2012). These complexes of SeqA could, in theory, physically block TnsE from accessing DNA during replication that could be vulnerable to transposition. This "protective" role for SeqA makes sense given the disadvantageous nature of transposon acquisition and other recombination events. Given SeqA's preference for binding hemimethylated GATC sites as well as to SeqA oligomers, it is proposed that regions rich in GATC sites are consequently rich in SeqA oligomers that inhibit TnsE access to the chromosome and therefore inhibit TnsE-mediated Tn7 transposition into SeqA bound DNAs (Li 2012).

SeqA, however, was not the only bacterial protein whose physiological role in TnsE-mediated transposition was studied. In 2006 a bioinformatic analysis by another group revealed a set of 18 genes (one of which was SeqA) that were highly

associated with the Dam gene (Brézellec 2006). In the Brézellec et al study, instead of focusing on complete genes and using a "bi-directional best hits" method, where genes are identified as best matches of each other, a protein domain-based strategy was used to generate the list of 18 genes that are "strictly confined" to genomes containing the Dam gene (Brézellec 2006). Given that *dam* and *seqA* mutants in prior research had been shown to have an effect on Tn7 transposition, it followed logically that the protein products of the other genes that were found to co-occur with *dam* could also potentially play a role in TnsE-mediated transposition.

Five genes were chosen from the set identified by Brézellec et al—*holD, metJ, mutH, ycbG (matP),* and *yfbV*—and studied in this project for a potential role in TnsE-mediated Tn7 transposition. HolD has a functional role as a DNA polymerase III loader in the DNA polymerase III holoenzyme (Kesseler 2011). Based on prior work revealing TnsE's recognition of the β-clamp processivity factor and HolD's role in the same holoenzyme, it was reasonable that HolD could possibly have an association with TnsE-mediated Tn7 transposition and was thus investigated. MutH was similarly an intriguing protein to further investigate given its role in the mismatch repair system that also functions on nascent DNAs. It is believed to be a weak endonuclease that identifies and cleaves the unmethylated strands at GATC complexes, precisely where our model predicts that SeqA occludes TnsE (domain sieve 2006, welsh et al. 1987). The presence of the SeqA complex at these GATC sites is believed to occur at the replication fork prior to MutH's function, allowing that an association between TnsE and MutH is possible.

YcbG, since renamed MatP, is likewise involved in the terminus region (Mercier 2008). It is a small DNA binding protein that associates with a signature motif of 13 bps found exclusively at the terminus region and, by keeping the Ter regions together, acts to prevent premature segregation of the new sister chromosomes (Dame 2011). MatP is a highly interesting protein to further study given its role in chromosome organization, and in particular because of its interaction with the Ter region, which TnsE-mediated Tn7 transposition appears to prefer. YfbV meanwhile has been found to limit the spreading of the MatP-DNA complex constraining the interaction to the terminus region of the *E. coli* chromosome during replication (Thiel, 2012). Tn7's tendency to transpose into the terminus region and YfbV's role in terminus "anchoring" gave reason for further studies on the potential of yfbV to have an effect on Tn7 transposition via the TnsE pathway. The remaining protein – MetJ, appears to be less directly related to a possible interaction with TnsE. MetJ acts as a transcriptional repressor of the Met regulon that codes for genes involved in methionine biosynthesis and transport (Augustus 2009). Nevertheless, repressor proteins have previously been shown to play important roles in apparently unrelated recombination reactions (Paul, 2004). PepA and ArgR, both transcriptional repressors, were found to be important in the maintenance of P1 Prophage and in doing so were involved in Cre-*lox* recombination *in vivo*. In addition to this, its association with *dam* and therefore *seqA* make MetJ a worthy protein to investigate.

There were a variety of objectives in this thesis. The first was to confirm that I could successfully repeat the *in vivo* Tn7 transposition assay in both wild-type and

seqA mutants with both the TnsABC* mutant core machinery, and more importantly, the TnsABC+E machinery. As was noted in prior work in the laboratory, any work with the *seqA* mutants must be approached with a great deal of care as they have a large tendency to easily pick up suppressors. With that in mind, the additional goal in carrying out this objective was to identify conditions that would not favor the acquisition of fast growing suppressors that would invalidate the experiment.

The second objective was to investigate whether SeqA plays a role in TnsE-mediated Tn7 targeting of mobile plasmids. This was to be performed by mapping out the location of Tn7 transposition in a TnsE-mediated pathway using genetic techniques. In order to map out the transposition events we would use to our advantage the fact that the TnsE-mediated pathway preferentially transposes Tn7 into conjugal plasmids at a proportion of ~95% (Wolkow 1996). This bias for transposition into plasmids could be associated with the difference between chromosomal replication, where replication of leading and lagging strand templates occur together, and conjugation, where replication of these templates occurs separately in the donor and recipient cells. Therefore in order to map out Tn7 transpositions following the *in vivo* transposition assay described earlier, we would use both "mating in" and "mating out" techniques in order to search the chromosomes and conjugal plasmids for a kanamycin cassette carried by our miniTn7 element (see Materials and Methods). Given our model then, we would expect that in a *seqA* mutant TnsE-mediated Tn7 transposition would occur at higher frequencies into the chromosome than in a wild type because SeqA would no

longer be there to block TnsE's access to the β-clamp processivity factor at the replication forks.

The third and final objective was to test for any specific effect of single gene knockouts of the *metJ, holD, mutH, yfbV,* and *matP* genes on *in vivo* transposition, specifically on the TnsE-mediated pathway. Given the functions of these genes' protein products and their co-occurrence with *dam* and therefore *seqA*, it is plausible that there exists a physical and/or functional interaction between these proteins and the TnsE-mediated transposition complex. This potential interaction would ostensibly be revealed in a simple *in vivo* transposition assay like that used for the *seqA* mutant. If a functional interaction was identified future work would look for determine if a this involved a direct physical interaction with TnsE or a secondary effect on process targeted by TnsE-mediated transposition.. Furthermore, it would be informative to identify any potential differences TnsE-mediated transposition known to target mobile plasmids transposition (i.e. observe whether Tn7 still heavily prefers the conjugal plasmids in these deletion strains) by performing both the mating in and mating out studies on the single gene knockout strains post Tn7 transduction, especially given the ease and low cost of mapping.

Materials and Methods

Strains, Media, and Antibiotics

A list of all of the strains used in this thesis is outlined in Table 1. LB and M9 Minimal media were prepared as described by Miller (1992). The following is a list of antibiotics used and their concentrations: streptomycin (Str), 50 μg/mL;

ampicillin (Amp), 100 μg/mL; chloramphenicol (Cam), 30 μg/mL; kanamycin (Kan), 50 μg/mL; tetracycline (Tet), 20 μg/mL; trimethoprim (Tmp), 100 μg/mL; nalidixic acid (Nal), 20 μg/mL; rifampicin (Rif), 50 μg/mL; and gentamycin (Gen), 10 μg/mL .

Introduction of Desired Mutations into Bacterial Strains

The 6 mutants were all constructed in the *E. coli* strain NLC28. Five of the mutants (*mutH, metJ, matP, and yfbV*) were ordered from the Keio Collection, coming in strains with backgrounds described in table 2, and isolated on LB + kanamycin media. The deletions were first isolated using the P1 *vir* Lysate protocol and then introduced into the NLC28 background using the P1 *vir* Transduction protocol, both outlined in Peters (2007). It is important to note that transductions were unsuccessful for the *holD* deletion and so therefore this deletion was not studied.

Removal of Kanamycin Resistance from Keio Collection Deletion Strains

In order to select for Tn7 transpositions in the *in-vivo* transposition assay, the strains need to be sensitive to kanamycin. The deletions isolated from the Keio Collection strains and transduced into the NLC28 strains, however, had a kanamycin marker that allowed for them to be selected. In order to remove the kanamycin resistance cassette flanked by FRT sites, the strains were first transformed with a temperature sensitive plasmid that coded for Flp-recombinase (Datsenko 2000). These strains were then incubated at high temperatures in order to both induce the expression of the Flp-recombinase gene as well as effectively eliminate the temperature sensitive plasmid due to its inability to replicate at high temperatures.

The Flp-recombinase, whose gene in this case was expressed at high levels at 42 degrees C, was essential for excision of the kanamycin marker. It operates via site-specific recombination by acting on the short flippase recognition target (FRT) sites flanking the marker, causing them to recombine out of the chromosome and consequently remove them from our strains due to their inability to replicate. Furthermore, it was essential that this plasmid's replication be temperature sensitive because it possessed a chloramphenicol resistance marker and the strains needed to be chloramphenicol sensitive in order for subsequent transformations with plasmids containing the Tn7 proteins to work properly. The temperature sensitive plasmid isolation, transformation and the 42 degree treatment protocols are outlined below.

Isolation of the Temperature Sensitive Plasmid

The temperature sensitive plasmid in this case was pCP20. It was isolated from an *E. coli* DH5α using the Promega© Pure Yield Plasmid Mini Prep System protocol and stored at -20 degrees C. A UV spectrophotometer was then used to determine the concentration of the plasmid via the dsDNA function and was found to be 34 ng/uL.

Transformation of the Keio Collection Deletion Strains with a Temperature Sensitive Plasmid

The NLC28 pOX-GenR strains each featuring one of the five deletions (*holD, metJ, matP, mutH, and yfbV*) were transformed with the pCP20 temperature sensitive plasmid according to the protocol outline in Peters (2007). The protocol

was modified to prevent loss of the temperature sensitive plasmid by adjusting all incubations to 30° C. Furthermore, it was also found to be beneficial to add kanamycin to the media in order to ensure that the deletions were maintained.

Removal of the antibiotic resistance cassette and curing the pCP20 plasmid

The transformants were streaked for individual colonies on LB plates, which were then incubated O/N at 42 degrees C. An individual colony from the 42 degree incubated LB plate was then streaked first onto an LB + Amp, then an LB + Kan plate, an LB + Gen plate, and finally streaked out for individual colonies again on an LB. The 3 LB plates with antibiotics were all incubated at 30 degrees C for two nights while the LB plate was incubated at 42 degrees O/N. This process was repeated once more in the event that the strains had not lost their pCP20 associated ampicillin resistance and their deletion marker kanamycin resistance.

Transformation of Deletion Strains with Plasmids Containing Transposition Proteins

The wild-type NLC28 as well as the 5 NLC28 deletions strains (*ΔseqA, ΔmatP, ΔmetJ, ΔyfbV, and ΔmutH*) were then each transformed according to the same previously outlined protocol with 4 different plasmids, yielding 20 different strain backgrounds. All of the incubation temperatures in the protocol, however, were adjusted from 30 degrees C to 37 because none of these 4 plasmids were temperature sensitive like the pCP20. Furthermore, transformants were selected for on LB+Cam+Gen plates for the Keio Collection deletion strains and LB+Cam for the Δ*seqA* strain.

The four plasmids all contained some combination of the transposition proteins used in the TnsE-mediated transposition pathway of Tn7. It was necessary to introduce these plasmids in order for the transposition proteins to be expressed *in-trans* during the *in-vivo* transposition assay, allowing for Tn7 transposition. The first plasmid (pCW15^{WT}) included only the genes for TnsA, B, and C, and was thus used as a negative control in that it lacked all of the proteins for the promotion of Tn7 transposition. The second plasmid (pCW15*) also included the genes for TnsA,B, and C. In this strain however, there was a gain of activity mutation in TnsC, TnsC^{A225V}, which allowed for the core machinery to stimulate successful transposition of Tn7 without the TnsE or TnsD protein. This plasmid thus served as a control for the effects of the particular allele on transposition itself, in so far as it allowed for the observation of Tn7 target selection without the presence of either target selection proteins (TnsE or D). The third plasmid (pJP123) included the genes for TnsA, B, C, and a a low expression orientation of *tnsE*, while the fourth plasmid (pJP124) also included the core transposition machinery TnsABC as well as a high expression orientation of *tnsE*. These latter two plasmids were used to observe the effects of TnsE on Tn7 target site selection. The low TnsE expression plasmid was studied in particular after initial trials of the *in-vivo* transposition assay revealed that there were significant differences in transpositions frequencies in this background as opposed to the high TnsE expression backgrounds.

Conjugation of the Transformed Deletion Strains

As stated earlier, prior research has suggested that the TnsE-mediated pathway promotes Tn7 transposition into actively conjugating plasmids. For this

reason it was necessary to mate in a conjugal plasmid into each of our deletion strains. Four of the deletions *(ΔmatP, ΔmetJ, ΔyfbV, and ΔmutH*) were introduced into an NLC28 strain already containing the pOX-Gen^R conjugal plasmid. All 8 (4 core machinery plasmid backgrounds per strain) NLC28 WT and the Δ*seqA* strains were mated with a CW51 donor strain that contains the pOX-Gen^R conjugal plasmid. This was accomplished by streaking out both the recipient and donor strains in a crosshatch fashion on an LB plate, and then selecting for exconjugants on an LB + Strep + Cam + Gen plate. Both the recipient and the donor strains were also checked on the selective plate as negative controls to ensure that conjugation was successful.

In-Vivo Transposition Assay ("λ Hop")

The λ hop assay was the central way of determining the effects the various deletions had on Tn7 transposition frequencies. As discussed earlier, this assay involved an integration and replication defective λ vector (λKK1 780 hisG9424::Tn10 del16 del17::attTn7::miniTn7::Kan^R) that was used to deliver a miniTn7 element to each of the deletion strain backgrounds. Successful transpositions were determined by the gain of kanamycin resistance since the miniTn7 involves a Kan^R marker flanked by Tn7 ends.

The assay involved first making 5 mL LB + 0.2% maltose + Cam O/N's of the WT strain and each of the 5 deletion strains with each of the 4 backgrounds. Initial trials of the protocol revealed that the Δ*seqA* strains yielded results more similar to those found in prior research using M9 Minimal Media + 5% LB instead of the LB media. The O/N's were then subcultured 1:100 and grown in a 37 degree C wheel

until their OD_{600} = 0.6. These were then centrifuged at 3500 rpm for 10 min, and their pellets re-suspended in 20 mM $MgSO_4$ until the OD_{600} = 2.0 (in order to ensure each were at equal concentrations). Then, in 10 trials per strain, 200 uL of strain was mixed with 200 uL of the λ KK1 phage lysate (titered to 2.7 x 10^8 PFU/mL), after which it was grown in a water bath at 37 degrees C for 15 min. At these two stages it was important to stagger the phage additions and water bath treatments into precise time intervals in order to equalize the amount of time that each sample incubated with the phage. After the water bath incubation, 1 mL of LB + 20 mM NaCitrate was added to each sample to inactivate remaining phage and they were grown in a 37 degree C wheel for 1.5 hours. Each sample was then spun down in the microcentrifuge for 30 sec at 16,000 xg, the supernatant poured off, and the pellet plated onto LB + NaCitrate + Kan plates. In addition, λ KK1 phage lysate and each strain were plated as negative controls. Transposition frequencies were then calculated by dividing the number of colonies that grew per plate by the number of infectious phage used. The protocols for the phage lysate preparation as well as titer calculation are outlined below.

Furthermore it must be noted that the protocol was adjusted for when the assay's results were meant to be analyzed by the mapping techniques described later. After the 15 min water bath incubation, 3 mL of LB + 20 mM NaCitrate was added to each aliquot. Then, this 3+ mL mixture of phage, strain, and LB + 20 mM NaCitrate was divided into 3 1 mL aliquots. From here the protocol was identical to the one described earlier.

Preparation of the λ KK1 Phage Lysate

The protocol was the same as for the P1 *vir* Lysate preparation with minor adjustments. The donor strain used here is a DH5α and the lysate added is the stock λ KK1 phage.

λ Phage KK1 Lysate Titer Determination

The phage titer was determined using the protocol outlined in Peters (2007). The titer was found to be 2.7 x 10^8 PFU/mL.

Mapping of the miniTn7 Transposition Events

As stated earlier, the primary objective of this research was to determine what effect SeqA, as well as the related bacterial proteins, have on Tn7 target site selection in TnsE-mediated transposition. Two genetic techniques were used to assess whether the Tn7 transposed into the pOX-Gen^R plasmid or the chromosomes of the deletion strains. A "mating-out" assay was performed, in which the pOX-Gen^R plasmid in the deletion strains was mated out and then screened for kanamycin resistance. Similarly a "mating-in" assay was also performed, in which another pOX-Tet^R, Tmp^R plasmid was mated into the deletions strains and then screened for the presence of kanamycin resistance.

"Mating-Out" Assay

A streak purified single colony from each of the λ hop trials from the low TnsE plasmid backgrounds was mated (in the cross hatch fashion described earlier) with a CW51 Nal^R Rif^R recipient strain. Exconjugants were selected for on LB + Nal +

Rif + Gen and these were then screened for kanamycin resistance on LB + Nal + Rif + Gen + Kan.

"Mating-In" Assay

A streak purified single colony from each of the λ hop trials from the low TnsE plasmid backgrounds was mated with a CW51 Nal^R Rif^R F-plasmid (Tn10:Tet^R Tn7:Tmp^R) donor strain. Exconjugants were selected for on LB + Cam + Tet + Tmp and these were then screened for kanamycin resistance on LB + Cam + Tet + Tmp + Kan.

Construction of Donor Strain for "Mating-In" Assay

Due to the fact that none of the stock F-plasmids featured a unique antibiotic that could be selected for, a novel one had to be created. First, an NLC28 $Strep^R$ attnTn7::Tn7 Tmp^R was mated with the donor strain XL1Blue F-plasmid (Tn10 = Tet^R). Exconjugants were selected for on LB + Strep + Tmp + Tet. These NLC28 exconjugants were then mated with the recipient strain CW51 Nal^R Rif^R, and their exconjugants were selected for on LB + Nal + Rif + Tet + Tmp. These exconjugants served as the donor strains in the "mating-in" assay.

Results

The data in Fig. 1 shows the results of a lambda hop assay performed on a WT strain as well as a *seqA* mutant, which, based on a comparison with data collected in prior work, likely picked up a suppressor. Clearly there is a significant decrease in transposition frequencies when comparing the *seqA* mutant strain with

a high TnsE expression plasmid to the WT counterpart. Although this may initially appear to conflict with the increase in Tn7 transposition frequencies seen in *seqA* mutants in prior work, it in reality is a false comparison as the data in Fig. 1 closely resembles that found when a *seqA* suppressor was acquired (Li 2012). Prior work revealed that *seqA* suppressors are less likely to occur when cells are in conditions like lower temperature or minimal media where they grow more slowly (Rotman, 2009). For this reason the lamba hop was later performed using cultures incubated at 30 degrees C. It was believed that incubation at this temperature that leads to a slower growth rate would be less selective against the *seqA* mutants and would thus decrease the chance of picking up suppressors. The 30 degree adjustment to the protocol, however, failed to eliminate the issue of suppressors, as seen in Fig. 2 where "*seqA* mutant" strains with a high TnsE expression plasmid had a lower transposition frequency than the WT high TnsE background.

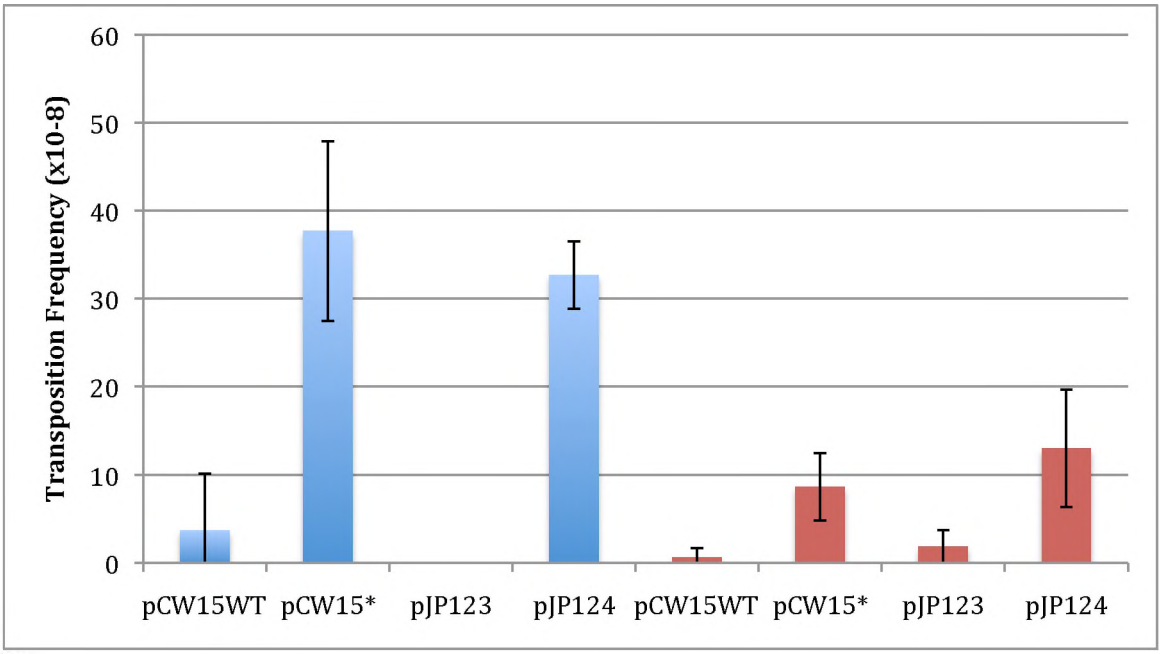

Fig. 1: Results of the preliminary lambda hop assay performed in an NLC28 WT background (blue) and in the *seqA* mutant (red), both featuring one of the 4 different plasmid backgrounds. pCW15^{WT} = TnsABC negative control, pCW15* = TnsABC^{A225V} positive control for general affects on Tn7 transposition (not specific to TnsE), pJP123 = TnsABC + a low expression of *tnsE*, pJP124 = TnsABC + a high expression of *tnsE*.

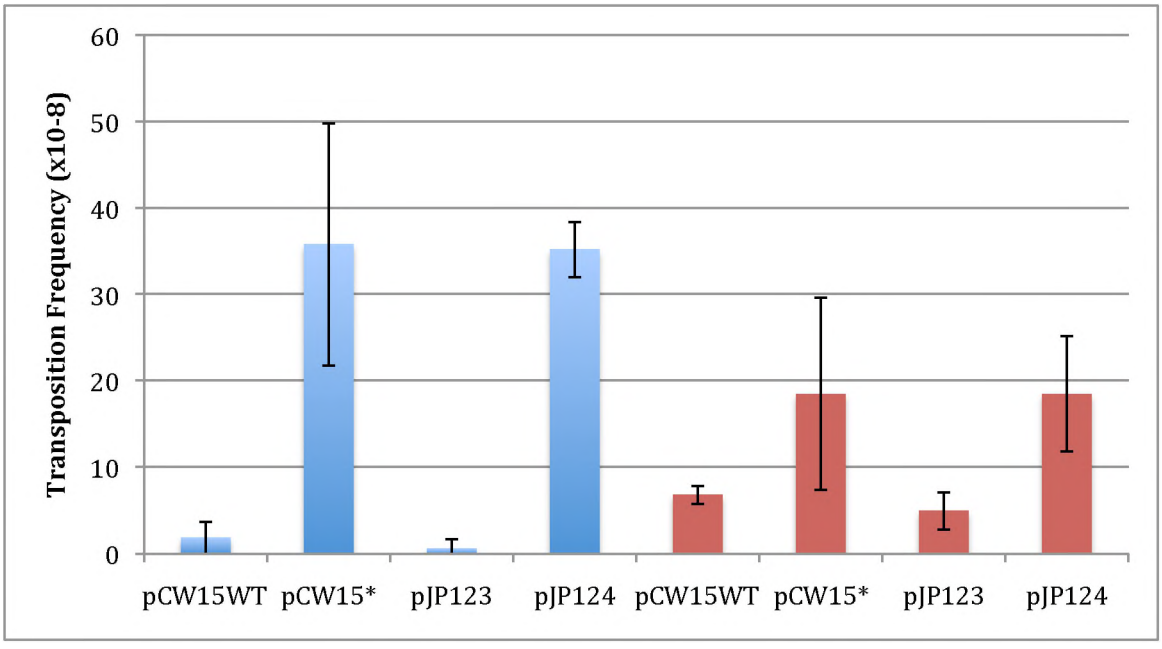

Fig. 2: Lambda hop performed with cultures prepared in LB media and incubated at 30 degrees C. An NLC28 WT background (blue) and a *seqA* mutant (red), both featuring one of the 4 different plasmid backgrounds, were assayed.

Another adjustment was made to the *in vivo* transposition assay protocol in an effort to make the growth conditions less selective against *seqA* mutants. This time the media was changed to M9 Minimal media which provides a less than ideal environment for growth than rich media (i.e. LB) and therefore slows down the growth rate, and in doing so decreases selection for suppressors. The M9 Minimal media proved impractical however, as the grow out phase following subculturing took too long to grow to an OD_{600} of 0.6. In order to speed up the process slightly, an M9 Minimal media with 5% LB was used, and the results from that lambda hop assay are outlined in Fig. 3. As the data in the figure illustrates, there was a clear 20-fold increase seen between the *seqA* low TnsE background and the WT. The lambda hop assay was repeated using the M9 Minimal + 5% LB media and adjusted for mapping purposes in order to reduce the likelihood of siblings (i.e. one transposition event that appears as two separate colonies). With this adjustment however, there was now only a 5-fold increase in transposition frequencies between the *seqA* low TnsE background and the WT (nonetheless significant).

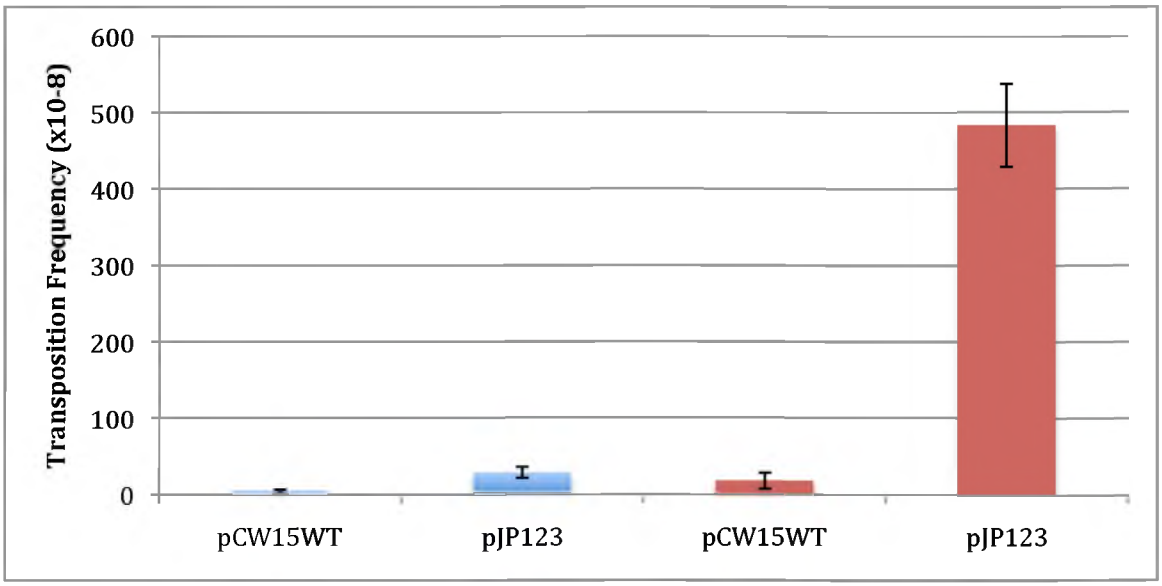

Fig. 3: Lambda hop performed with cultures prepared in M9 minimal + 5% LB media. An NLC28 WT background (blue) and a *seqA* mutant (red), both featuring the wild type ABC core machinery only (pCW15^{WT}) or the TnsABC + TnsE expressed at low levels (pJP123), were assayed.

The lambda hop assay was then repeated on the Δ*metJ*, Δ*matP*, Δ*mutH*, and Δ*yfbV* single gene knockout strains, all featuring either the mutant core machinery positive control (pCW15*) or the TnsABC + low E expression vector (pJP123). Δ*holD* could not be assayed because the deletion strain could not be constructed into our strain background. This was attempted several times and could be explained by the importance of HolD to the cell, especially given how sick the *holD* deletion cultures appeared to be. The *holD* was not examined further because of the low viability of this strain. The data in Fig. 4 reveals that none of the deletions caused an increase in transposition frequencies like that seen in the *seqA* mutant. Furthermore, it appears that, when compared to the WT pCW15* background, transposition frequencies in the strains with the TnsABC* mutant core machinery increased 3-fold in the *metJ* mutant, 4-fold in the *matP* mutant, and 6-fold in both the *mutH* mutant and the *yfbV*

mutant. A further lambda hop assay, this time adjusted for mapping purposes (see Materials and Methods), was performed on the *metJ* and the *yfbV* mutants with the low TnsE plasmids. While the transposition frequency decreased moderately but not quite significantly in the *yfbV* mutant, it appears to have decreased significantly in the *metJ* mutant (transposition frequency of 0 for all trials). The results however, rule out the possibility that Tn7 transposition is unable to occur in *metJ* mutants, as there is a clear stimulation in transposition frequency (compared to the WT background) in the *metJ* mutant with a TnsABC* mutant core machinery. It remains possible that the *metJ* product may be required for TnsE-mediated transposition although confirming this would require examining transposition in strains that produce greater amounts of TnsE and other follow-up experiments.

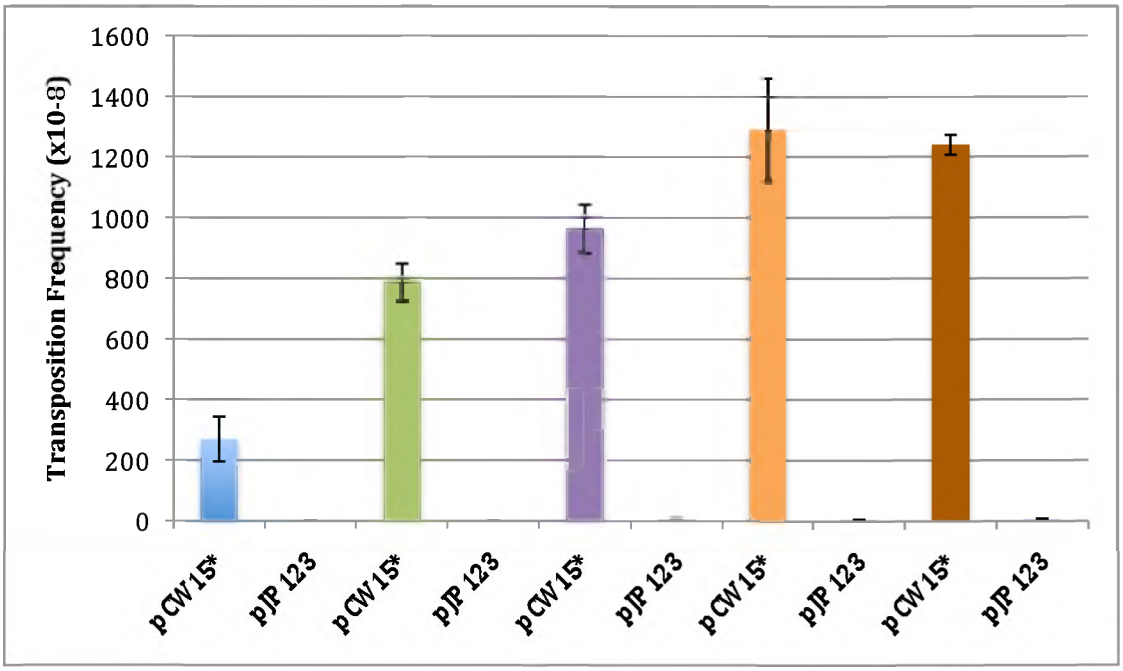

Fig. 4: Lambda hop performed in the Keio Collection deletion strains and in an NLC28 WT control (blue). The deletions assayed were Δ*metJ* (green), Δ*matP* (purple), Δ*mutH* (orange), and Δ*yfbV* (brown), all featuring either the mutant TnsABC* (pCW15*) or TnsABC + low expression of E (pJP123) core machinery expressed *in trans*.

Finally, colonies obtained from the lambda hop assays were streak purified and subjected to the mating in and mating out assays (see Materials and Methods). Both assays essentially screen for the kanamycin resistance that was introduced into the cell's genome via the miniTn7 delivered by the lambda phage. The mating in assay reveals whether the chromosome has gained a kanamycin marker (suggesting the miniTn7 elements transposed into it), while the mating out assay reveals whether the pOX-Gen^R conjugal plasmid has gained a kanamycin marker (suggesting the miniTn7 elements transposed into it). As the data in table 3 reveals, the miniTn7 preferentially transposes into the conjugal plasmid when it utilizes the TnsE-mediated pathway in a WT background. In 90% of cases in the mating out assay the kanamycin marker was found on the pOX-Gen plasmid while the mating in assay

reveals that the marker was found within the chromosome in only 10% of trials. In the *seqA* mutant, however, the data reveals that the kanamycin marker was found on the conjugal plasmid in only 40% of trials while it was found in the chromosome in 60% of trials.

There was an unusual result for the *seqA* isolates 3 and 7 as they showed up as kanamycin sensitive in the mating in assay and unable to conjugate in the mating out assay (Table 4, Supplemental Information). Here the kanamycin resistance was believed to have been lost upon curing the pOX-Gen plasmid by replication exclusion in the "mating in" assay, but could not be mated out with gentamycin selection, suggesting that the miniTn7 transposed into the conjugal plasmid. Such an event remains a reasonable possibility given the large proportion of the plasmid dedicated to conjugation genes. By knocking this ability out, the pOX-Gen plasmid would be unable to mate out, explaining why exconjugants could not be isolated from the mating out assay of those isolates. Furthermore, in the *yfbV* mutant the kanamycin marker transposed into the pOX-Gen plasmid in 33% of cases while it transposed into the chromosome in 67% of cases. It must be noted however that the mapping data for the *yfbV* mutant comes from only 6 trials, versus the 30 for the WT strain and the 10 for the *seqA* mutant.

Table 3: Results from both the mating in and mating out assays performed on strains after delivery of the miniTn7 Kanamycin marker via the lambda hop assay. The percentages show the proportion of trials, associated with a location in the genome, where kanamycin resistance was found. The locations of the kanamycin marker were determined by a comparison of the results from the complementary mating in and out assays (see Table 4, Supplementary Information).

Strain Background	Number tested	Chromosome	Conjugal plasmid
NLC28 WT pJP123	30	10%	90%
ΔseqA pJP123	10	60%	40%
ΔyfbV pJP123	6	67%	33%

Discussion

The results presented in this thesis offer a variety of new insights into the objectives discussed as well as avenues for further investigation. The basic goal was to confirm that I could successfully repeat the *in vivo* Tn7 transposition assay, presented by Li in his dissertation, in both wild-type and *seqA* mutants. It was important to reach this goal in order to then map out the assay results and it was also a difficult goal to achieve given the ease with which suppressors were acquired. Additionally, I planned to extend this work to give insight about any changes in targeting to conjugal plasmids in a Tns-E mediated Tn7 transposition pathway. As in prior work, there was a recurring issue of suppressor acquisition in the *seqA* mutants (Fig. 1 and 2), as well as in the faster growth rates during subculturing, monitored via OD_{600} measurements. I identified conditions that did not favor the acquisition of suppressor mutations, allowing me to get consistent results (Fig. 3). A *seqA* deletion mutant was successfully isolated that gave consistent results by using an M9 minimal + 5% LB media. Prior work suggesting a 10-fold increase in Tn7

transposition in a TnsE pathway system was then substantiated when a 20-fold increase in TnsE-mediated Tn7 transposition was observed (Fig. 3) (Li 2012).

Some caution is warranted in my results as only a 5-fold increase in transposition frequencies was seen in the trials used for mapping. Although it was a significant change, it leaves open the possibility that suppressors were still able to accumulate even under the slow growth conditions. The drop in frequency could be the result of a variety of factors. For example, the adjustment of the protocol for mapping purposes resulted in many more tubes, which may have increased the incubation times possibly at critical steps. Also, these strains were reconstructed with the *seqA* deletion allele, which could have allowed the acquisition of a new suppressor mutation. Furthermore, repeated measurement of the phage titer revealed that it was susceptible to significant decreases in PFU's/mL, which would obviously affect the calculated transposition frequency.

Once a consistent method was established, a further investigation was carried out studying the effects the SeqA-TnsE interaction has on TnsE-mediated target site selection (chromosomal or conjugal site) in *in* vivo transposition. Based on the results obtained by others in their exploration of the physical interaction between the two proteins and the resulting functional role of that interaction, a model was developed whereby SeqA binds at the replication fork and in doing so was believed to block TnsE's access to the DNA and the β clamp processivity factor involved in lagging strand synthesis. Given the adverse effects that the accumulation of transposons generally yields, it makes sense that bacteria like *E. coli* would have

evolved a mechanism like that of SeqA binding at the replication fork that could occlude Tn7 and other mobile elements like it (perhaps even host mediated recombination) that utilize the lagging-strand template as a target for recombination. In order to see if SeqA was having an effect on TnsE-mediated Tn7 site selection, causing it to favor transposition into conjugal plasmids, the Tn7 events were mapped using genetic techniques in a *seqA* mutant.

The consolidation of the results from the complementary mating in and mating out assays was used to determine whether Tn7 transposed into the chromosome or into the pOX-Gen conjugal plasmid in a TnsE-mediated pathway. The mapping data for the WT background illustrated in Table 3 largely supports the results found in prior research. In the past, Tn7 was found to preferentially transpose into conjugal plasmids at a proportion of ~95% when utilizing the TnsABC+E machinery. The results of the mating out assay revealed that in 90% of the transposition events mapped, the kanamycin marker was found on the pOX-Gen conjugal plasmid, while the results of the mating in assay revealed that in 10% of the transposition events mapped, the kanamycin marker was found on the chromosome. A comparison of these two results suggests that the miniTn7 element transposed into the conjugal plasmid in 90% of trials, and therefore supports the original findings where there was a preference for TnsE-mediated transposition for the pOX-Gen plasmid (~95%).

The deviation from the original findings in both assays is likely a result of the small sampling population assayed. In the mating out assay there exists the

possibility that the miniTn7 element could transpose into either the Gen cassette or the genes required for conjugation, thus yielding an isolate incapable of being screened because of the lack of ability to generate exconjugants that would then be screened for Kan^R.

The mapping data from the assays performed on the *seqA* mutant and presented in Table 3 suggests that the SeqA-TnsE interaction has an effect on Tn7 target site selection. The kanamycin marker was found on the pOX-Gen plasmid in only 40% of events mapped using the mating out assay and on the chromosome in 60% of events mapped using the mating in assay. Given the assumption that kanamycin markers were obtained via transposition of the miniTn7 element, the consolidation of these two data suggests that in the *seqA* mutants, Tn7, via the TnsE pathway, transposed into the conjugal plasmid at much lower frequencies than in the WT background. In other words, it shows that Tn7 transposed into the chromosome at a significantly higher frequency in cells unable to produce SeqA. Thus, the data is consistent with the idea that TnsE had much more access to the chromosomal DNA in the absence of SeqA, thus lending support to our proposed model. Naturally both assays were limited in the same ways as they were in WT strains discussed above. Furthermore, there was a discrepancy between the complementary assays because isolates 3 and 7 appeared to be kanamycin sensitive in both assays (Table 4). The mating in assay data for these two isolates shows that the kanamycin marker was not on the chromosome while the mating out assay showed that these isolates could not act as donors in conjugation. A comparison of this data then suggests that Tn7 transposition did not occur into the chromosome,

but rather into the genes required for conjugation on the conjugal plasmids. This hypothesis is supported by the inability of those isolates to mate out their conjugal plasmids in the mating out assay and their inability to grow on kanamycin in the mating in assay.

The results from the investigation of the set of DNA replication proteins that co-occur with SeqA in strains with ancestral *dam* genomes brought about new insights into their effects on TnsE-mediated Tn7 transposition and consequently their potential interaction with TnsE. As stated before, a *holD* mutant could not be efficiently isolated, likely due to the importance of HolD to DNA replication (although it has been shown to be non-essential in an MG1655 background), and therefore could not be studied further. The results from the *in vivo* transposition assay performed on the remaining four deletion strains - Δ*metJ*, Δ*matP*, Δ*mutH*, and Δ*yfbV* – showed no increase in Tn7 transposition in the TnsE background (Fig. 4), and actually showed a significant decrease in the *metJ* mutant. Curiously, in the TnsABC* mutant core machinery backgrounds, Tn7 transposition increased 3-fold in the *metJ* mutant, 4-fold in the *matP* mutant, and 6-fold in both the *mutH* mutant and the *yfbV* mutant (Fig. 4). The data suggests that the basic functions underlying Tn7 transposition generally increase in the absence of these proteins but yet the TnsE-mediated system may be reliant on them, at the very least in the case of MetJ. The only significant change in TnsE-mediated transposition frequency occurred in the *ΔmetJ* mutant, where no successful Tn7 transpositions were observed in the TnsE mediated pathway within the detection of our assay. Given that Tn7 was found to be able to function in a *ΔmetJ* mutant in the positive control (TnsABC*) strain the

role of the MetJ remains intriguing but unproven. The data from the *yfbV* gene were too small to make any substantive conclusions, but could suggest that the *yfbV* system may have a role in targeting transposition into conjugal plasmids. Further research will be needed to substantiate any affect of this gene on TnsE-mediated transposition.

The results discussed all present a variety of opportunities for further investigation. Primarily, the mapping data for the *seqA* mutants featuring the TnsABC+E machinery, and therefore our model, could be strengthened by increasing the amount of transposition events mapped and utilizing a more dependable technique for mapping insertions. For example, an arbitrary PCR technique can be used to determining the exact location of the miniTn7 by amplifying a substrate that can be sequenced (Peters and Craig 2001). Furthermore, lambda hop assays should be performed on larger samples of the Δ*metJ*, Δ*matP*, Δ*mutH*, and Δ*yfbV* strains in both the TnsABC* mutant and TnsE core machinery backgrounds. If a true functional interaction can be found with the above alleles, future work could involve performing protein-protein interaction assays between TnsE and each of the proteins outlined above in a similar fashion to how the physical interaction between SeqA and TnsE was discovered. Alternatively, if a process seems to be targeted, future work would involve determining what that process is, for example conjugation or some processing event that occurs on the DNA template strand that undergoes lagging-strand DNA synthesis. Finally, it would be worthwhile to identify whether an interaction exists between TnsE and the other proteins that were found to be co-occurring with SeqA. Ultimately the goal of the

previous, present, and future work performed on the Tn7 transposon is to better understand bacterial replication as well as the role of this uniquely specific transposon in bacterial evolution.

Supplemental Information

Table 1: Strains used in this study

Strain	Genotype	Reference
MC4100	F- *araD139 Δ(argF-lac) U169 prsL150 relA1 deoC1 fthD5301 pstF25 rbsR22 e14- (fimB-fimE)632::IS1 (fruK-yeiR)725*	Cassadaban et al, 1976
NLC28	MC4100 ValR	McKnown et al, 1987
JP1386	NLC28 *Δara714*	Peters, J. E., et al, 2001
AP7	NLC28 *ΔseqA*::Tet^R	Laboratory stock
AC054	JP1386 *ΔholD::*Kan^R	This work
AC055	JP1386 *ΔmetJ::*Kan^R	This work
AC057	JP1386 *ΔmutH::*Kan^R	This work
AC056	JP1386 *ΔmatP::*Kan^R	This work
AC058	JP1386 *ΔyfbV::*Kan^R	This work
OH1	attTn7::$Tn7^{WT}$ (Tmp^R)	Laboratory stock
XL1Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB* $lacI^qZ\Delta M15$ Tn10 (Tet^R)]	Bullock, W.O., et al. 1987
CW51	F- *ara- arg- lacpro*XIII *recA*56 Nal^R Rif^R	Waddell et al 1988
DH5α	F- *λ- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80 dlacZ ΔM15 Δ(lacZYA-argF)U169, hsdR17(rK- mK+)*	Laboratory stock

Table 2: Backgrounds of the Strains from which Deletions were Isolated

Mutation	Function	CGSC#	Designation	Source	Genotype
holD	DNA pol III loader	11091	JW4334-4	Keio Collection	F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514, **ΔholD767::kan**
metJ	Met Apo-repressor	10823	JW3909-1	Keio Collection	F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, **ΔmetJ725::kan**, hsdR514
mutH*	DNA mismatch repair	10186	JW2799-1	Keio Collection	F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, **ΔmutH756::kan,** rph-1, Δ(rhaD-rhaB)568, hsdR514
ycbG/matP	Unknown	12061	JW0939-1	Keio Collection	F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, **Δ matP771::kan**, rph-1, Δ(rhaD-rhaB)568, hsdR514
yfbV	Unknown	9842	JW2292-1	Keio Collection	F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, **ΔyfbV777::kan**, rph-1, Δ(rhaD-rhaB)568, hsdR514

Table 3: Plasmids used in this study

Plasmid	Description	Reference
pCW15^{WT}	Cm^R; TnsABC cloned in pACYC184 vector	Peters, et al 2000
pCW15*	Cm^R; TnsABC* cloned in pACYC184 vector	Stellwagen, et. al. 1996
pJP123	Cm^R; TnsABC+low expression of E cloned in pACYC184 vector	Peters, et al 2000
pJP124	Cm^R; TnsABC+ high expression of E cloned in pACYC184 vector	Peters, et al 2000
pOX-G	Gen^R ; F plasmid	Stellwagen, et. al. 1996
pCP20	Cm^R Amp^R; Temperature sensitive plasmid with thermal inducible FLP recombinase	Datsenko, et. al. 2000

Table 4: Results from both the "mating in" and "mating out" assays performed on the isolates from the mapping adjusted lambda hop performed on the NLC28 WT, *seqA* mutant, and *yfbV* mutant strains, all with TnsABC + low expression of *tnsE*

Strain Background	Isolate	Result of Mating In Assay, Chromosome was Kan^R	Result of Mating Out Assay, pOX-Gen was Kan^R	Interpretation based on both assays.
NLC28 pJP123	1	-	+	Plasmid
	2	+	-	Chromosome
	3	-	+	Plasmid
	4	-	+	Plasmid
	5	-	+	Plasmid
	6	-	+	Plasmid
	7	-	+	Plasmid
	8	-	+	Plasmid
	9	-	+	Plasmid
	10	-	+	Plasmid
	11	-	+	Plasmid
	12	+	-	Chromsome
	13	-	+	Plasmid
	14	-	+	Plasmid
	15	+	-	Chromosome

	16	-	+	Plasmid
	17	-	+	Plasmid
	18	-	+	Plasmid
	19	-	+	Plasmid
	20	-	+	Plasmid
	21	-	+	Plasmid
	22	-	+	Plasmid
	23	-	+	Plasmid
	24	-	+	Plasmid
	25	-	+	Plasmid
	26	-	+	Plasmid
	27	-	+	Plasmid
	28	-	+	Plasmid
	29	-	+	Plasmid
	30	-	+	Plasmid
NLC28 *ΔseqA* pJP123	1	-	+	Plasmid
	2	+	-	Chromosome
	3	-	-	Plasmid*
	4	-	+	Plasmid
	5	+	-	Chromosome
	6	+	-	Chromosome

	7	-	-	Plasmid*
	8	+	-	Chromosome
	9	+	-	Chromosome
	10	+	-	Chromosome
JP1386 *ΔyfbV*	1	-	+	Plasmid
	2	+	+	Unresolved**
	3	+	-	Chromosome
	4	+	-	Chromosome
	5	-	+	Plasmid
	6	+	-	Chromosome
	7	+	-	Chromosome

* When the Kanamycin resistance was lost upon curing the pOX-Gen plasmid by replication exclusion in the "Mating in" assay, but could not be mated out with Gentamycin selection, the assumption was that the miniTn7 can was on the conjugal plasmid.

** When the Kanamycin resistance was not lost upon curing the pOX-Gen plasmid by replication exclusion in the "Mating in" assay, but could also be mated out with Kanamycin selection, thus it was impossible to determine the initial location.

References

Augustus, A. M., Reardon, P. N., and Spicer, L. D. (2009). MetJ repressor interactions with DNA probed by in-cell NMR. Proc. Natl. Acad. Sci. U S A , 106(13): 5065 – 5069.

Baba, T., T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, H. Mori (2006). Construction of *E. coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. 2:1-11.

Brendler, T., J. Sawitzke, K. Sergueev, and S. Austin. (2000). A case for sliding SeqA tracts at anchored replication forks during Escherichia coli chromosome replication and segregation. Embo. J., 19:6249-58.

Brézellec, P., Hoebeke, M., Hiet, M.-S., Pasek, S., & Ferat, J.-L. (2006). DomainSieve: a protein domain-based screen that led to the identification of dam-associated genes with potential link to DNA maintenance. Bioinformatics, 22(16): 1935–1941. doi:10.1093/bioinformatics/btl336.

Dame, R. T., Kalmykowa, O. J., Grainger, D. C. (2011). Chromosomal macrodomains and associated proteins: implications for DNA organization and replication in Gram negative bacteria. PLoS Genetics, 7(6): ISSN 1553-7390.

Datsenko, K. A., and B. L. Wanner. (2000). One-step inactivation of chromosomal genes in *E. coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U S A 97:6640-5.

Jones, R. N. (2005). McClintock's controlling elements: the full story. Cytogenet. Genome Res. 109:90-103.

Keseler, I. M., et, al. (2011) EcoCyc: a comprehensive database of *E. coli* biology. Nucleic Acids Research, 39 (Database issue): D583 - D590. ISSN 0305-1048.

Li, Z. (2012). *A molecular mechanism allowing transposon Tn7 to target active DNA replication* (Doctoral dissertation). Cornell University.

Li, Z., Craig, N. L, Peters, J. E. (2013) Transposon *Tn7*. In A. P. Roberts & P. Mullany (eds.), *Bacterial Integrative Mobile Genetic Elements.* Portland, OR: Landes Bioscience.

Lu, M., J. L. Campbell, E. Boye, and N. Kleckner. (1994). SeqA: a negative modulator of replication initiation in *E. coli*. Cell, 77:413-26.

Miller, J. H. (1992). A short course in bacterial genetics: a laboratory manual and handbook for *E. coli* and related bacteria, Vol. 1. New York, NY: CSHL Press.

Onogi, T., H. Niki, M. Yamazoe, and S. Hiraga. (1999). The assembly and migration of SeqA-Gfp fusion in living cells of *E. coli*. Mol. Micro. 31:1775-82.

Parks, A. R., Zaoping L., Qiaojuan S., Roisin M. O., Moonsoo M. J., and J. E. Peters (2009). Transposition into replicating DNA occurs through interaction with the processivity factor, Cell, 138 (4): 685-695. ISSN 0092-8674, 10.1016/j.cell.2009.06.011.

Peters, J. P., and Craig, N. L. (2000). Tn7 transposes proximal to DNA double-strand breaks and into regions where chromosomal DNA replication terminates. Molecular Cell, Vol. 6: 573–582.

Peters, J. P., and Craig, N. L. (2001). Tn7 recognizes transposition target structures associated with DNA replication using the DNA-binding protein TnsE. Genes & Dev. 15: 737-747. doi:10.1101/gad.870201.

Peters, J. E. 2007. Gene transfer - Gram-negative bacteria. In C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. Marzluf, T. M. Schmidt, and L. R. Snyder (ed.), Methods for General and Molecular Microbiology, 3 ed. ASM Press, Washington D.C.

Roberts, D., B. C. Hoopes, W. R. McClure, and N. Kleckner (1985). IS10 transposition is regulated by DNA adenine methylation. Cell 43:117-30.

Rotman, E., Bratcher, P. and Kuzminov, A. (2009). Reduced lipopolysaccharide phosphorylation in *E. coli* lowers the elevated ori/ter ratio in *seqA* mutants. Mol. Micro. 72(5): 1273-1292.

Stellwagen, A. E., and N. L. Craig. (1997). Gain-of-function mutations in TnsC, an ATP-dependent transposition protein that activates the bacterial transposon Tn7. Genetics, 145:573-85.

Sunirmal Paul, David Summers (2004). ArgR and PepA, accessory proteins for XerCD-mediated resolution of ColE1 dimers, are also required for stable maintenance of the P1 prophage. Plasmid 52(1): 63-68. ISSN 0147-619X, 10.1016/j.plasmid.2004.04.003.

Thiel, A., Valens, M., Vallet-Gely, I., Espeli, O., & Boccard, F. (2012). Long-range chromosome organization in *E. coli*: a site-specific system isolates the Ter macrodomain. PLoS genetics, 8(4), e1002672. doi:10.1371/journal.pgen.1002672

Waddell, C. S., and N. L. Craig. 1988. Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes. Genes Dev. 2:137-49.

Wolkow, C. A., R. T. DeBoy, and N. L. Craig. (1996). Conjugating plasmids are preferred targets for Tn7. Genes Dev., 10: 2145-57.

Yin, J. C., M. P. Krebs, and W. S. Reznikoff. (1988). Effect of dam methylation on Tn5 transposition. J Mol. Biol., 199: 35-45.

Acknowledgements

I would like to acknowledge all of the people who have helped and encouraged me throughout this honors thesis project. First and foremost Dr. Joseph Peters for giving me the chance to explore my interests in genetics in his laboratory. He has been a great mentor and guide throughout my project, and his patience with me and passion for the subject made this a project a memorable experience from which I have learned that I want to become a researcher. I would also like to thank Ashwana Fricker from the Peters' lab for her willingness to help me whenever I was having trouble and for always being a caring friend. Barbara Eaglesham who introduced me to microbiology in a lab setting and more importantly supported and encouraged me throughout my project. My parents, Helena and Mario Chramiec, who always demanded the best from me throughout my years in school and who taught me the importance of critical thinking. Yakov and Margarita who have been there for me whenever I needed them. Finally, I would like to thank Maria Minsker, who continues to be my best friend and an incredible partner.