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SetR_{ICE391}, a negative transcriptional regulator of the integrating conjugative element 391 mutagenic response

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Abstract

The integrating conjugative element ICE391 (formerly known as IncJ R391) harbors an error-prone DNA polymerase V ortholog, polV_{ICE391}, encoded by the ICE391 *rumAB* operon. polV and its orthologs have previously been shown to be major contributors to spontaneous and DNA damage-induced mutagenesis *in vivo*. As a result, multiple levels of regulation are imposed on the polymerases so as to avoid aberrant mutagenesis. We report here, that the mutagenesis-promoting activity of polV_{ICE391} is additionally regulated by a transcriptional repressor encoded by SetR_{ICE391}, since *Escherichia coli* expressing SetR_{ICE391} demonstrated reduced levels of polV_{ICE391}-mediated spontaneous mutagenesis relative to cells lacking SetR_{ICE391}. SetR_{ICE391} regulation was shown to be specific for the *rumAB* operon and *in vitro* studies with highly purified SetR_{ICE391} revealed that under alkaline conditions, as well as in the presence of activated RecA, SetR_{ICE391} undergoes a self-mediated cleavage reaction that inactivates repressor functions. Conversely, a non-cleavable SetR_{ICE391} mutant capable of maintaining repressor activity, even in the presence of activated RecA, exhibited low levels of polV_{ICE391}-dependent mutagenesis. Electrophoretic mobility shift assays revealed that SetR_{ICE391} acts as a transcriptional repressor by binding to a site overlapping the –35 region of the *rumAB* operon promoter. Our study therefore provides evidence indicating that SetR_{ICE391} acts as a transcriptional repressor of the ICE391-encoded mutagenic response.

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Author contributions

MG contributed to the design of the study, participated in the writing of the manuscript, created the non-cleavable SetR_{ICE391}, and performed mutagenesis assays on the polV orthologs; DH cloned, expressed and purified native SetR_{ICE391}, as well as performed *in vitro* cleavage reactions and *in vivo* mutagenesis assays; MPM performed electrophoretic mobility shift gel assays; JPM provided phylogenetic analysis of SetR_{ICE391} and related proteins, made plasmids pJM1300, pJM1317 and pJM1318 and performed *in vivo* mutagenesis assays; AMG cloned SetR_{ICE391}, performed the initial mutagenesis assays and contributed to the design of the study; KSB performed the mutagenesis assay on the non-cleavable SetR_{ICE391} mutants and contributed to the design of the study; RW contributed to the design of the study and helped write the manuscript.

Conflict of interest

The authors report no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.dnarep.2018.11.007>.

Keywords

SOS response; Integrating conjugative element; Mutagenesis; DNA polymerase V; ICE391

1. Introduction

DNA damaging agents cause DNA replication blocking lesions that induce the expression of multiple genes whose gene products act to minimize the effects of the damage through DNA repair and, following extensive damage, DNA damage tolerance mechanisms. In *Escherichia coli* these mechanisms form the SOS response and their regulation is dependent on the interplay of the LexA and RecA proteins. LexA is the negative transcriptional regulator of more than 40 SOS response genes that function in DNA repair, mutagenesis, and the regulation of cell division [1–3]. As the representative member of the clan SF serine peptidases, LexA possesses a catalytic serine-lysine dyad capable of executing self-cleavage at the Ala84-Gly85 bond within LexA [4,5]. The process of LexA self-cleavage relies on an interaction with RecA. After DNA damage, DNA polymerase III encounters a DNA lesion that it is unable to traverse and dissociates from the damaged strand and reinitiates further downstream. The resulting single-stranded DNA gap is bound by RecA creating a nucleoprotein filament that is often referred to as RecA*. LexA binds avidly to RecA* [6] and in doing so, undergoes a self-cleavage reaction that inactivates LexA's ability to serve as a transcriptional repressor and leads to the induction of the SOS response [7,8].

The SOS response is structured so as to react to a range of DNA damage by first initiating the expression of genes capable of processing DNA damage in an error-free manner, such as the genes responsible for nucleotide excision repair. Following extensive DNA damage, the error-free response is overwhelmed dictating expression of genes that make-up the mutagenic aspect of the SOS response. It is the affinity of LexA for its binding site, or LexA box, upstream of each SOS-regulated gene which allows for the temporal control of the SOS response; the higher affinity of LexA for the LexA box, the greater damage necessary for expression of the associated gene(s) [1,2]. Of the 40 or so LexA-regulated genes, three encode the catalytic subunits of DNA polymerases, polII (*polB*); polIV (*dinB*); and polV (*umuC*). All three polymerase have been shown to facilitate translesion DNA synthesis (TLS) [9–13]. polII and polIV generally do so in an error-free manner [11,14–17]. However, polV-dependent TLS is generally error-prone and is the major contributor to induced mutagenesis after DNA damage [18,19].

polV is a multi-subunit DNA polymerase. It is composed of two subunits of UmuD', the RecA*-stimulated autodigestion product of UmuD [20–22], and one subunit of UmuC (UmuD'₂C) [23,24]. However, polV appears to have limited catalytic activity, at least *in vitro* [25]. The catalytic activity of polV is, however, dramatically stimulated in the presence of RecA* and ATP [25,26]. It is believed that a RecA molecule and ATP are transferred from RecA* to polV to form a higher order structure termed "polV Mut" (consisting of UmuD'₂C-RecA and ATP) that is the functionally and mutagenically active form of the enzyme that facilitates error-prone TLS [26–28].

Orthologs of *umuDC*, such as *mucAB* and *rumAB*, are also negatively regulated by LexA, require RecA*-stimulated self-cleavage of the UmuD-like protein and entail an interaction of the UmuD'- and UmuC-like proteins in a stoichiometry resembling UmuD'₂C (and probably RecA and ATP), in order to perform TLS [29–31]. Unlike the chromosomally encoded *umuDC* operon, *mucAB* and *rumAB* are readily transferred between species as components of mobile genetic elements. *rumAB* movement between species is mediated *via* the Integrative Conjugative Element (ICE), ICE391 (formerly known as IncJ R391), and much like the conjugative plasmid, R46, which harbors *mucAB* [32,33], it also includes the transfer of drug resistance genes. The *rumAB* operon is of unique interest because of the weak *rumAB*-mediated mutator activity promoted by the native ICE391 [34,35] when compared to the robust spontaneous and induced mutation frequency of the sub-cloned *rumAB* [29,31,36].

ICE391 is a member of the SXT/R391 family of ICEs [37]. All ICEs exist integrated into the host chromosome and encode all the genes necessary for conjugation and integration/excision, as there is no circular replicative form like that seen with conjugative plasmids. SXT and ICE391 are each composed of approximately 90 kb of DNA and are closely related elements demonstrating 95% nucleotide identity over 65 kb of DNA [38]. Although a greater knowledge of SXT biology exists, the degree of identity between these ICEs, for the most part, allows inference that ICE391 likely acts in a related manner. For example, regulation of the genes involved in conjugation, integration and excision are highly conserved between SXT and ICE391. The amino acid sequence of the SXT regulators SetC, SetD and SetR and the surrounding regulatory DNA sequences are 95–100% identical to those of ICE391. SetR_{SXT} (this designation will be used to differentiate SetR_{SXT} from the identical SetR_{ICE391}) is a homolog of the λ cI-like repressor proteins and retains the amino acids needed for self-cleavage in the presence of RecA*. It is therefore not surprising that induction of the SOS response inactivates SetR_{SXT} repressor function, allowing for the expression of SetC and SetD, which are activators of conjugation, excision and integration [39,40]. SetR_{SXT} also appears to auto-regulate its own expression in a manner similar to the λ cI repressor [41]. This exit strategy, during times of stress, is reminiscent of prophage excision from the host chromosome after DNA damage [42].

ICE391 harbors the LexA-regulated *rumAB* operon, which is responsible for the production of the DNA polV ortholog, Ruma'₂B (polV_{ICE391}) [31]; however, the limited mutability of the native ICE391 compared to the sub-cloned *rumAB* operon [31,34,35], implicates other *trans*-acting regulatory factors, possibly encoded on ICE391, beyond the host LexA protein.

Our research has identified SetR_{ICE391} as a strong candidate for the transcriptional repression of polV_{ICE391} expression. Based on the role of SetR_{SXT} as a transcriptional repressor of *setCD*, and its homology to the cI-like repressors, including LexA, and the conserved nature of the operators bound by LexA and those bound by SetR_{SXT}, we investigated whether SetR_{ICE391} might be involved in the negative regulation of the ICE391 encoded *rumAB* operon.

2. Materials and methods

2.1. Bacterial strains and plasmids

Bacterial strains and plasmids used in this study are listed in Table 1. The SetR_{ICE391} expressing plasmid, pAMG1, was constructed by amplifying *setR* via PCR from ICE391 with the primers SetRNdeI5' (5'-ATC CAC ATA TGA AAA CTT TAT CCG AAC G-3', the *setR* start codon is in bold and the NdeI site is underlined) and SetRBamHI3' (5'-AAA GGA TCC CTC TCA GCG TGC GCC AAT GC-3', BamHI site underlined) (Sigma). The resulting ~730bp DNA fragment was digested with NdeI and BamHI and the *setR*_{ICE391} fragment was cloned into the similarly digested vector pMal-p2X (New England Biolabs) to form the IPTG-inducible SetR_{ICE391} producing plasmid pAMG1. The SetR_{ICE391} over-producing plasmid, pDH9, was generated by synthesizing an *E. coli* codon optimized *setR*_{ICE391} gene (Genscript) and sub-cloning the synthesized gene into NdeI and XhoI restriction enzyme sites of pET22b+ (EMD Millipore). Plasmids pRW154 (*umuDC*), pRW144 (*mucAB*) and pRW290 (*rumAB*) have been described elsewhere [36,43]. pRLH421 is a low-copy number plasmid harboring an ~ 21.5 kb partial *EcoRI* digest of *episomal* ICE391 (Genbank [U13633](#)). A 493 bp PmeI to Bsu36I *setR*_{ICE391} gene fragment (Genscript) encoding the SetR_{ICE391}(G94R) non-cleavable variant was sub-cloned into pRLH421 to generate pJM1300. pJM1317 and pJM1318 were constructed by cloning either the wild-type *setR*_{ICE391} gene, or the *setR*_{ICE391}(G94R) gene (Genscript) into pRW290 from PstI to BamHI.

Where noted, the following antibiotics were used for selection; Zeocin (25 µg ml⁻¹), Tetracycline (15 µg ml⁻¹), Spectinomycin (50 µg ml⁻¹) and Ampicillin (100 µg ml⁻¹).

2.2. Qualitative analysis of spontaneous reversion of the *hisG4(oc)*

The *E. coli* strains were transformed with plasmids expressing SetR_{ICE391} (pRLH421, pJM1317, pAMG1), the non-cleavable SetR_{ICE391}(G94R) (pJM1300, pJM1318), RumAB (pRW290) or the cloning vector (pMal-p2X). The data reported in Fig. 3 also included using compatible low copy, spectinomycin resistant plasmids expressing UmuDC (pRW154) or MucAB (pRW144) to assess the specificity of SetR_{ICE391} activity. Three antibiotic resistant isolates from each transformation were grown in LB medium containing the proper antibiotics overnight at 37 °C. Five hundred microliters of each overnight culture were centrifuged and the pellet suspended in an equal volume of SM buffer. Next, spontaneous mutagenesis was determined by plating 100µl in triplicate on Davis and Mingioli minimal agar plates [44] plus glucose (0.4% w/v); agar (1.0% w/v); proline, threonine, valine, leucine, and isoleucine (all at 100 µg ml⁻¹); thiamine (0.25 µg ml⁻¹); and containing a trace amount of histidine (1 µg ml⁻¹). Plates were incubated at 37 °C for three days. The results presented in Figs. 2, 3 and S1 represent the average number of His⁺ mutants from nine plates per cultured transformant (± standard error of the mean [SEM]).

2.3. Visualization of cellular levels of RumA

A 1:100 dilution in fresh LB medium, plus appropriate antibiotics, of the overnight cultures used in the spontaneous reversion of *hisG4(oc)* assay (see above) were incubated at 37 °C until they reached early exponential phase. A 1.0 ml aliquot of each culture was centrifuged,

and the resulting cell pellet was suspended in 4 x SDS sample buffer [40% glycerol (v/v), 9.2% SDS (w/v), 4 mM DTT, 250 mM Tris–HCl (pH 6.8), and 0.2% (w/v) bromophenol blue]. Equal protein concentrations of the aliquots were determined using the Pierce BCA Protein Assay (ThermoFisher Scientific) and electrophoresed on 12% SDS-polyacrylamide gels. Next, proteins were transferred to an Immobilon P membrane (Millipore) and subsequently incubated with polyclonal antibodies directed against the RumA protein (Covance). Visualization of the transferred RumA protein was performed on Kodak BioMaxMR film using the BioRad Immun-Star AP substrate.

2.4. Phylogenetic analysis of transcriptional repressors

A multiple sequence alignment of the core region of the SetR protein from ICE391 and various other homologous proteins were performed using the ClustalW algorithm in MacVector (version 14.0.6). The alignment was exported from MacVector as a Phylip file. This Phylip file was imported into the SplitsTree4 (version 4.14.4) and an unrooted phylogenetic tree was generated by setting the distance method to BioNJ.

2.5. Expression and purification of native SetR_{ICE391}

The ICE391 SetR protein was overproduced using the T7 protein expression system (EMD Millipore). Overproduced SetR_{ICE391} was purified using protocols previously developed for the purification of LexA and DinR proteins [45,46]. Strain RW1290 harboring pDH9 was grown overnight at 37 °C in LB broth containing 100 µg ml⁻¹ ampicillin. Cells were diluted 100-fold in LB broth containing ampicillin and grown at 37 °C. At an OD_{600 nm} of approximately 0.5, IPTG was added to a final concentration of 1 mM, and growth continued at 37 °C for an additional 2 h, after which time cells were harvested by centrifugation at 2600 x *g* for 10 min at 4 °C. The cell pellet was resuspended in RS buffer [50 mM Tris (pH 7.8) and 10% w/v sucrose] in 1/25 the original culture volume and stored at –80 °C. Cells were slowly thawed on ice and lysed *via* sonication. The resulting material was centrifuged at 185,500 x *g* for 1 h. The supernatant was collected, and ammonium sulfate added to a final concentration of 35% (w/v). The precipitate was centrifuged at 185,500 x *g* for 30 min and the supernatant was collected. Ammonium sulfate was then added to the supernatant to a final concentration of 50% (w/v). The precipitate was centrifuged, and the pellet resuspended in buffer B [20 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, 10% glycerol (v/v), and 1 mM DTT] containing 250 mM KCl and dialyzed against the same buffer overnight. Immediately before loading on a phosphocellulose column (Whatman, Clifton, NJ), the dialysate was diluted in buffer B to a final concentration of 100 mM KCl. The phosphocellulose column was washed with two column volumes of buffer B containing 100 mM KCl and SetR_{ICE391} was eluted with a linear gradient of 100–500 mM KCl in buffer B. Fractions containing SetR_{ICE391} (as judged by visual inspection of Coomassie-stained gels) were pooled and applied to a hydroxyapatite column (Bio-Rad, Hercules, CA). The column was washed with two column volumes of buffer C [0.1 mM EDTA, 10% glycerol (v/v), and 1 mM DTT] containing 50 mM KHPO₄ (pH 7.0). SetR_{ICE391} was eluted with a linear gradient of 50–300 mM KHPO₄ in buffer C. Fractions containing highly purified SetR_{ICE391} were pooled and stored at –80 °C after increasing the glycerol concentration to 20% (v/v).

2.6. SetR_{ICE391} in vitro cleavage reactions

Autodigestion reactions of purified SetR_{ICE391} occurred at 37 °C in 50 mM CAPS-NaOH (pH 10.0). Each reaction mixture (50 µl) contained 3 µg of SetR_{ICE391}. Reactions were terminated at the appropriate times by the addition of 4 x SDS sample buffer and rapidly freezing the samples on dry ice.

RecA-mediated cleavage of SetR_{ICE391} was based upon a previously described procedure for the RecA-mediated cleavage of UmuD [21]. Standard reaction mixtures (50 µl) contained 10 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 0.1 µM UTT48 DNA oligo (5'-TCG ATA CTG GTA CTA ATG ATT AAC GAA TTA AGC ACG TCC GTA CCA TCG-3'), 1 mM ATP γ S, 2 µg of purified *E. coli* RecA (New England Biolabs), and 3 µg of SetR_{ICE391}. Reactions were incubated at 37 °C for the appropriate times and terminated by adding 4 x SDS sample buffer and freezing on dry ice.

The products of the autodigestion and RecA-mediated cleavage reactions were subsequently subjected to electrophoresis in SDS-polyacrylamide gel electrophoresis (PAGE) gels containing 4–12% polyacrylamide. Proteins were visualized after staining gels with Coomassie brilliant blue R-250.

2.7. Electrophoretic mobility shift gel assays

The 123 bp region upstream of SetR_{ICE391} was generated by annealing two complementary synthetic oligonucleotides, corresponding to the intergenic regions between the *croS*_{ICE391} and *setR*_{ICE391} genes (Fig. 6A). A 194 bp PCR amplicon containing the SetR_{ICE391} binding site (s) upstream of the *rumAB* operon was generated using primers RumF; 5'-GCG AGC TAT CCC CAC ATC TA-3' and RumR; 5'-CTA CGG CCT ATC AGC GAG AC-3' (Genscript, Piscataway, NJ) and the *rumAB* operon-containing plasmid, pRW290, as a template. Similarly, the SetR_{ICE391} binding site mutant used in Fig. 7, was initially synthesized as a 205 bp EcoRI-HindIII fragment (Genscript) that was cloned into pUC57 and subsequently amplified using the RumF and RumR PCR primers. DNAs were 5' end labeled with [γ -³²P] ATP (Perkin Elmer, 3000 Ci/mmol) using T4 polynucleotide kinase (Roche). The purity of the probes was analyzed on a 20% polyacrylamide gel. Labeled probes had an average specific activity of 2000 cpm/fmol of DNA.

DNA-binding assay and DNA band-shifts were modifications of the method used in Winterling et al., 1996 [46]. Reaction mixtures containing labeled probe (~10,000–30,000 cpm µl⁻¹) and purified SetR_{ICE391}, or LexA protein, ranging from 0 to 200 nM were incubated at room temperature for 25 min in binding buffer (150 mM NaCl, 20 mM Tris-HCl [pH 7.5], 0.2 mM EDTA, 1.0 mM MgCl₂, 5% glycerol [v/v], 50 µg ml⁻¹ bovine serum albumin [BSA], 1 mM DTT) with a final reaction volume of 20 µl each. Binding assays were performed multiple times with and without polydIdC as a competitor of nonspecific binding. However, we found that the presence or absence of polydIdC did not influence the outcome of the results. These results were expected, since the EMSAs were performed with purified proteins, rather than cell extracts [47]. Protein-DNA complexes were separated in native polyacrylamide gels (6% acrylamide). Gels were dried and subsequently exposed to Kodak Biomax XAR film for appropriate periods of time.

3. Results

3.1. Phylogenetic relationship of SetR_{ICE391} to λ cl-like repressors

Characterization of the ICE391 *rumAB* operon identified a DNA polV ortholog that belongs to an evolutionary distinct subfamily of the polV-like Y-family polymerases [29]. Relative to the other cloned polV orthologs characterized, polV_{R391} demonstrates a higher frequency of spontaneous mutagenesis and transversion mutations [31]. polV_{ICE391} also promotes much higher induced mutation frequencies than that found for MucA'2B (polR1), another member of the polV-like family, that has historically played a major role in increasing the sensitivity of the “Ames Test” used to detect carcinogens [48]. Interestingly, the high mutation frequency of polV_{ICE391} is only evident with the sub-cloned operon and is not seen with the native ICE391 [31,34,35].

ICE391 is a mobile genetic element capable of transfer between different species of bacteria; therefore, it is plausible that ICE391 carries factors necessary to minimize aberrant polV_{ICE391}-mediated mutagenesis. We chose to address the potential role of SetR_{ICE391} in limiting expression of the *rumAB* operon since SetR_{ICE391} is identical at the amino acid level to the repressor of SXT excision and transfer, SetR_{SXT} [38].

The λ -like family of transcriptional repressors are represented by chromosomally encoded members, as well as by those carried on mobile genetic elements. Phylogenetic analysis generated from multiple alignments of the core amino acid sequences of the λ -like repressor sequences separated the chromosomal *E. coli* LexA and *B. subtilis* DinR proteins from the bacteriophage λ -like proteins and SetR_{ICE391} (Fig. 1A). Indeed, SetR_{ICE391} was more closely aligned with the bacteriophage λ imm434 λ I and P22 λ c2 repressors (Fig. 1A). All transcriptional repressors subject to RecA-mediated self-cleavage, including SetR_{ICE391}, possess a conserved alanine-glycine cleavage site and a serine-lysine dyad responsible for carrying out the activated RecA-mediated self-cleavage reaction (Fig. 1B). As with the identical SXT SetR protein, SetR_{ICE391} is predicted to possess the amino-terminal helix turn helix DNA binding domain of the λ -like repressors [40].

3.2. Expression of *rumAB*-dependent mutagenesis is LexA regulated

Ideally, the role of SetR_{ICE391} in *rumAB*-dependent mutagenesis would be best studied in the context of the entire ICE391. However, previous work on SXT revealed manipulation of *setR_{SXT}* in a cell harboring SXT was deleterious to the host cell [39]. In order to address the role of SetR_{ICE391} in regulating *rumAB*-dependent mutagenic activity, we utilized pRLH421, a plasmid construct containing an ~21.5 kb fragment of ICE391 cloned into the low-copy number plasmid, pGB2 [36]. The ~88.5 kb ICE391 normally integrates into the 5' end of the *prfC* gene in the *E. coli* genome [49]. *setR_{ICE391}* is located at the very 3' end of the linear ICE391 (bp 87561–88208). However, it is evident that pRLH421 was generated by a partial *EcoRI* digest of *episomal* ICE391, since the ~21.5 kb ICE391 insert contains sequences from both the 5' (bp 1–20241) and 3' (bp 87297–88532) ends of the linear ICE391 and includes numerous open reading frames including the *rumAB* operon and *setR_{ICE391}* (Fig. 2 A).

To assess the mutagenic capacity of the *rumAB* operon in pRLH421, the levels of spontaneous mutagenesis were measured *via* a qualitative mutagenic assay that measures the reversion of the *hisG4* ochre allele [43]. The *rumAB*-mediated mutagenic activity in the strains expressing different *lexA* alleles was consistent with LexA-regulated *rumAB* expression. The number of histidine revertants per plate was greatest in the strain lacking a functional LexA [*lexA51*(Def)] while the strain expressing a non-cleavable LexA protein [*lexA3*(Ind⁻)] produced the fewest histidine revertants per plate (Fig. 2C). Because the wild-type *lexA*⁺ strain maintains a low basal rate of cleavage, the number of histidine revertants was intermediate of that seen for the *lexA51*(Def) strain and the non-cleavable *lexA3*(Ind⁻) strain (Fig. 2C). LexA regulation of the *rumAB* operon is therefore consistent with the identification of a potential LexA-binding site in the promoter region of the *rumAB* operon [29].

3.3. A non-cleavable G94R mutant of SetR_{ICE391} abrogates *rumAB*-dependent mutagenesis in a *recA718 lexA*(Def) strain

Interaction of RecA with single stranded DNA formed following a DNA damaging event activates RecA (RecA*) thus facilitating the self-cleavage of repressors, such as the *E. coli* LexA repressor and *B. subtilis* DinR repressor, thereby leading to the induction of the SOS response [45,50]. RecA*-mediated self-cleavage has also been described for the prophage repressors of numerous temperate bacteriophage [7,51,52]. Although non-cleavable mutants of these repressors span the entire gene sequence, a substantial number of these mutants impact the cleavage site, or the catalytic serine-lysine dyad [53,54]. To determine if SetR_{ICE391} cleavage is required for derepression of *rumAB*, we constructed a plasmid similar to pRLH421, designated pJM1300, expressing a mutant of SetR_{ICE391} at the highly conserved cleavage site of the transcriptional repressors that we predicted would render the protein non-cleavable. Based on the inducible minus mutations previously characterized in the bacteriophage λ cI repressor [53], we changed the Gly94 residue to Arg94 (G94R). The SetR_{ICE391}(G94R) expressing plasmid was then transformed into strains expressing the different *lexA* alleles described above. As expected, the pattern of mutagenic activity for the SetR_{ICE391}(G94R) expressing plasmid mimics that of the wild-type SetR_{ICE391} (Fig. 2C).

Inactivation of SetR_{ICE391} was demonstrated in the *E. coli* strain MVG114 [*recA718 lexA51*(Def) *umuDC596::ermGT hisG4*]. The *recA718* allele produces an activated RecA* protein when expressed in a LexA defective background, or following DNA damage to the cell [55]. Due to the proficient co-protease activity of RecA718 and possible inactivation of the SetR_{ICE391} protein, plasmid encoded SetR_{ICE391} is incapable of reducing RumAB activity in the *recA718* strain (Fig. 2D). This was in dramatic contrast to the non-cleavable SetR_{ICE391}(G94R) mutant, which is largely insensitive to the activated RecA718 protein, and exhibited reduced levels of *rumAB*-dependent spontaneous mutagenesis (Fig. 2D). These observations support the hypothesis that SetR_{ICE391} cleavage is required for maximal *rumAB* expression and polV_{ICE391} mutagenesis-promoting activity.

Given the phylogenetic relationship of SetR_{ICE391} to λ cI-like transcriptional repressors, we considered the possibility that SetR_{ICE391} might help attenuate expression of the RumAB proteins. To address this issue, we examined the intracellular levels of RumA expressed in

the presence and absence of SetR_{ICE391} (Supplementary Fig. 1A, B). Indeed, intracellular levels of RumA were substantially lower in the presence of SetR_{ICE391} than in its absence (Supplementary Fig. 1A, B). Therefore, in the presence of SetR_{ICE391} the intracellular levels of RumA (and most likely RumB as well) are reduced, which in turn, impacts the extent of *rumAB*-mediated spontaneous mutagenesis.

The ~21.5 kb DNA fragment from ICE391 cloned into plasmids pRLH421 and pJM1300 include numerous open reading frames, in addition to the *rumAB* operon and *setR*_{ICE391} gene, that may influence polV_{ICE391} mutagenesis-promoting activity. In order to definitively assess the role of *setR*_{ICE391} in *rumAB* expression, we constructed low-copy plasmids that were devoid of these multiple open reading frames (Fig. 2B). Expression of either *setR*_{ICE391} (pJM1317), or *setR*_{ICE391}(G94R) (pJM1318) in the presence of the *rumAB* operon, as well as expression of *rumAB* in the absence of *setR*_{ICE391} (pRW290, see [43]) leads to comparable numbers of histidine revertants in both *recA*⁺ *lexA*⁺ and *recA*⁺ *lexA*Δ(*Ind*⁻) strains (Fig. 2E). However, the impact of SetR_{ICE391} on polV_{ICE391} mutagenesis-promoting activity is fully evident in the *recA*⁺ *lexA*Δ51(Def) strain. The presence of *setR*_{ICE391} or *setR*_{ICE391}(G94R) leads to a greater than four-fold reduction in histidine revertants compared to the plasmid expressing just *rumAB* (Fig. 2E). Moreover, expression of these plasmids in a *recA*718 *lexA*Δ51(Def) strain further reinforces the idea that cleavage of SetR_{ICE391} is required for maximal *rumAB* expression (Fig. 2F).

3.4. SetR_{ICE391} down-regulates ICE391-encoded *rumAB*, but not related polV-orthologs

Characterization of SetR_{SXT} involvement in the regulation of SXT excision and transfer previously identified four comparable 14-bp SetR_{SXT} binding sites with partial dyad symmetry, each containing an 8-bp AT-rich spacer region. Indeed, SetR_{SXT} binding at the four operators inhibits expression from the P_L promoter responsible for the transcription of *setCD*, which are activators of SXT excision and transfer, and from the P_R promoter for *setR*_{SXT} [40,41]. Therefore, the reduction in spontaneous mutagenesis evident in the presence of SetR_{ICE391} (Fig. 2D) is most likely the result of SetR_{ICE391} repressor activity upon the regulatory region upstream of *rumAB*. We addressed the specificity of SetR_{ICE391} for the plasmid encoded *rumAB* operon (pRW290) by measuring the level of spontaneous mutagenesis of the *E. coli umuDC* (pRW154) and *mucAB* (pRW144) operons in the *recA*⁺ *lexA*Δ51(Def) (*umuDC*)596::*ermGT hisG4* background (RW570). Analysis of the levels of spontaneous mutagenesis promoted by polV_{ICE391}, *E. coli* polV and R46 encoded polRI, reveals that SetR_{ICE391} specifically down-regulates only ICE391-encoded *rumAB* activity and has minimal effect on the ability of either polV or polRI to promote spontaneous mutagenesis *in vivo* (Fig. 3).

3.5. SetR_{ICE391} undergoes self-cleavage in the presence of RecA or under alkaline conditions

SetR_{ICE391} possesses the necessary amino acid residues to undergo a RecA*-mediated self-cleavage reaction (Fig. 1B). The fact that plasmid encoded SetR_{ICE391} could only suppress *rumAB*-mediated mutagenesis in a *recA*⁺ strain, and not in a *recA*718 strain, is a strong indicator that like LexA and cI-like proteins, SetR_{ICE391} is also subject to a RecA-mediated cleavage reaction. We wanted to directly test this hypothesis and to do so we first

overproduced and purified the native (untagged) SetR_{ICE391} protein in *E.coli*, using the methodology and protocols (see Materials and methods) that were previously used to purify *E.coli* LexA and *B.subtilis* DinR proteins [2,45]. Using this strategy, SetR_{ICE391} was purified to greater than 95% homogeneity (Fig. 4).

The highly purified SetR_{ICE391} was then incubated with RecA, single-stranded DNA as a polynucleotide cofactor, and ATP γ S as a mononucleotide cofactor in an *in vitro* cleavage reaction (Fig. 5). Reactions were terminated at different time points and samples separated by SDS-PAGE and visualized after staining the gel with Coomassie Blue dye. As early as two hours into the reaction the SetR_{ICE391} C-terminal fragment generated by the RecA-mediated self-cleavage at the Ala-Gly cleavage site begin to appear and by 24 h almost the entire SetR_{ICE391} sample had been cleaved (Fig. 5). We can therefore conclude that SetR_{ICE391} does indeed undergo a RecA*-mediated cleavage reaction.

The LexA and *cI*-like repressors also possess the ability to undergo RecA-independent autocatalytic cleavage when incubated at alkaline conditions [7]. To determine if the SetR_{ICE391} cleavage is mechanistically similar to LexA and *cI*-like proteins, self-cleavage of the purified SetR_{ICE391} protein was assayed in the absence of RecA* at pH 10.0. Under these conditions, LexA undergoes rapid self-cleavage, whilst that of λ *cI* is much slower [7]. Considering the closer phylogenetic relationship of SetR_{ICE391} to the *cI*-like proteins than to the LexA-like repressors (Fig. 1A), it was not surprising that we observed minimal pH-dependent self-cleavage of SetR_{ICE391} even after 24 h incubation at pH 10.0 (Fig. 5). The limited pH dependent auto-cleavage therefore firmly places SetR_{ICE391} in the *cI*-like family of transcriptional repressors, rather than the LexA-like repressors.

3.6. Binding of SetR_{ICE391} to sites upstream of setR_{ICE391} and the ICE391 rumAB operon

Reduced levels of *rumAB*-mediated spontaneous mutagenesis in the presence of SetR_{ICE391} (Fig. 2), coupled with the RecA-mediated self-cleavage of SetR_{ICE391} (Fig. 5) strongly indicate the involvement of SetR_{ICE391} in the transcriptional repression of the mutagenic SOS response of the ICE391. The discovery that the primary amino acid sequence of the SetR_{SXT} protein is identical to that of SetR_{ICE391}, prompted us to examine the DNA region upstream of the *rumAB* operon for possible SetR_{ICE391} binding sites. Based on the previously described SetR_{SXT} binding sites (Fig. 6A) [41], we identified an operator sequence partially overlapping the putative-35 promoter region of the *rumAB* operon (Fig. 6B). We were therefore interested in assaying whether highly purified SetR_{ICE391} physically interacts with the region upstream of *rumAB* (Fig. 6C). First, we confirmed that the purified SetR_{ICE391} protein would bind to sites upstream of the *setR_{ICE391}* gene, similar to that previously reported for *setR_{SXT}* [40]. To do so, we synthesized two 123bp oligonucleotides which when annealed to each other would be identical to the intragenic region between *croS_{ICE391}* and *setR_{ICE391}* of ICE391 (Fig. 6A). This intragenic region is identical between ICE391 and SXT and was previously identified in SXT to contain four SetR_{SXT} binding sites [40,41]. As clearly seen, based upon differential retardation of the ³²P-labeled DNA probe, SetR_{ICE391} appears to bind to a minimum of three discrete sites on the probe (Fig. 6C), and this observation is consistent with the previous results of SetR_{SXT} binding to the same DNA region on SXT [40].

To determine if SetR_{ICE391} physically interacts with the upstream regulatory region of *rumAB*, we used two primers to PCR amplify a 194bp amplicon spanning the upstream promoter region of *rumAB* including the very 5' region of *rumA* (Fig. 6B) and the ³²P-labelled 194-bp substrate was incubated with increasing concentrations of SetR_{ICE391} protein. Weak binding of SetR_{ICE391} was observed at a protein concentration of 10 nM (Fig. 6C). As the SetR_{ICE391} protein concentration increases beyond 10 nM, the initial band intensifies, and additional gel retardation occurs suggesting that there may be an additional SetR binding site located within the 194 bp probe (Fig. 6C)

Only one LexA binding site has been identified upstream of the *rumAB* operon [29] (Fig. 6B), and as expected, only one retarded band is observed when the *rumAB* probe was incubated with purified *E.coli* LexA protein (Fig. 6C).

3.7. Identification of the specific SetR_{ICE391} binding site upstream of the *rumAB* operon

In order to unequivocally determine the SetR_{ICE391} binding site(s) upstream of the *rumAB* operon, DNase I footprint analysis was under-taken as a custom contract service by scientists at Profacgen (Shirley, NY). DNase I footprint analysis in the presence and absence of highly purified SetR_{ICE391} with the *rumAB* promoter/operator sequence identified two distinct protected areas that coincide with the proposed SetR_{ICE391} binding site that overlaps the -35 promoter region (Figs. 6C and Fig. 7A). No additional sites that are protected by SetR_{ICE391} were identified by DNase I footprinting, suggesting that the “super-shift” of the probe observed at high concentrations of SetR_{ICE391} in Fig. 6C, is likely due to oligomerization of the SetR_{ICE391} on the single SetR_{ICE391} binding site located in the -35 region of the *rumAB* promoter. We then created mutations within the two half-sites of the SetR_{ICE391} binding site and utilized EMSA to determine SetR_{ICE391} specificity for the binding-site mutant (Fig. 7A). Alteration of the protected nucleotides of the *rumAB* -35 promoter region equivalent to the most conserved nucleotides of the SetR_{SXT} consensus sequence resulted in a loss of affinity of SetR_{ICE391} even at concentrations of the protein which gave a substantial shift of the wild-type DNA sequence (Fig. 7B). SetR_{ICE391} binding within the *rumAB* promoter region, combined with the data presented above (Figs. 2 & 3), is therefore consistent with SetR_{ICE391} acting as a transcriptional repressor of the *rumAB* operon.

4. Discussion

The mutagenic SOS response in *E.coli* is regulated at multiple levels [56]. The first key step relies on the RecA*-mediated self-cleavage and inactivation of the LexA transcriptional repressor to derepress expression of LexA-regulated proteins. The *E.coli umuDC* operon has a LexA-binding site with high homology to the consensus-binding site [2] and as a consequence, the UmuD and UmuC proteins are induced late in the SOS response. The *rumAB* operon, encoding polV_{ICE391}, also possesses an upstream LexA-binding site, but it differs from the consensus LexA-binding site at two additional positions, suggesting that LexA-dependent transcriptional regulation of *rumAB* might not be as vigorous as that observed for *E.coli umuDC*. We therefore considered the possibility that expression of *rumAB* might be subject to alternate levels of regulation, thereby keeping the mutagenesis

promoting activity the RumA[']B-encoded polV_{ICE391} to a minimum. We present evidence here that SetR_{ICE391} provides one such level of regulation.

Unlike *E. coli* polV, or R46-encoded polRI, SetR_{ICE391} specifically functions as an additional transcriptional repressor of the polV_{ICE391} polymerase. Indeed, alignment and phylogenetic analyses of the core amino acid sequences of SetR_{ICE391} and its homologs identified SetR_{ICE391} as a member of the α -like family of transcriptional repressors, which includes LexA (Fig. 1). Expression of SetR_{ICE391} in an *E. coli* *recA*⁺ *lexA51*(Def) strain results in reduced levels of *rumAB*-directed spontaneous mutagenesis (Fig. 2). In contrast, there was little negative regulation in a *recA718* strain expressing RecA* in the *lexA51*(Def) strain (Fig. 2D & F). This observation can be explained by the fact that purified SetR_{ICE391} was shown to undergo RecA*-mediated cleavage *in vitro* (Fig. 5). We assume that such processing occurs rapidly in a *recA718* strain *in vivo* thereby abrogating the ability of SetR_{ICE391} to serve as a transcriptional repressor of the *rumAB* operon. This hypothesis is supported by the observation that *rumAB*-dependent mutagenesis was greatly reduced in a *recA718* strain in the presence of a non-cleavable SetR_{ICE391} variant (G94R) (Fig. 2D & F). It is important to note that the *recA718* *lexA51*(Def) background more closely mimics the physiological state of a *recA*⁺ cell following extensive DNA damage suggesting that the ability of SetR_{ICE391} to down-regulate the *rumAB* operon after DNA damage may also be limited.

The level of genetic diversity seen within a species of bacteria is absolutely dependent on the process of horizontal gene transfer [57]. The movement of genetic material between organisms can provide an advantage to the recipient cell; one of the most clinically relevant examples is the acquisition of antibiotic resistance genes. Another mechanism capable of imparting resistance to antibiotics is the error-prone nature of the members of the Y-family of DNA polymerases. Because of its mobility, and with the identification and characterization of the error-prone polV_{R391}, it has become apparent that ICE391 not only factors in the establishment of antibiotic resistance but possibly influences the overall genetic diversity of the integrated ICE391 and the host organism.

ICEs are found integrated within the genome of many species of the γ -Proteobacteria. It was in an isolate of *Providencia rettgeri*, formally known as *Proteus rettgeri*, that ICE391 (originally identified as the IncJ plasmid, R391) was first identified [58]. Although SXT and ICE391 both carry the genes necessary for a mutagenic SOS response, an SXT-mediated mutagenic SOS response is lacking due to an IS_{CR2} element insertion in *rumB* [38]. The broad range transmissibility of ICE391 poses a compelling question: how is the *rumAB*-mediated aberrant mutagenesis minimized in these varied bacterial genetic backgrounds? The identification of a LexA box within the promoter region of the *rumAB* operon linked regulation of the mutagenic capacity of ICE391 with the host LexA repressor. While LexA-like regulation is widely distributed amongst bacteria, it is not universally present in all bacteria [59,60]. Furthermore, not all host LexA homologs are expected to bind the *rumAB* LexA-box with sufficient affinity, or at all, to adequately repress the ICE391 mutagenic SOS response. For instance, employing a broad host range plasmid encoding an *E. coli* *recA-lacZ* fusion, a wide variety of gram-negative microorganisms were found to be capable of repression and induction of the *lacZ* fusion based on the DNA damage state of the cell [61];

however, it was also demonstrated that the more distantly related the organism, the less affinity the LexA-like repressors possess for the *E. coli recA* LexA box.

Identification of SetR_{ICE391} as an SOS-induced repressor of *rumAB* reveals that ICE391 harbors a self-contained regulatory mechanism to curtail the mutagenic SOS response. Induction of the SetR regulon (*setCD*, *setR* and *rumAB*), probably functions to maximize the continued proliferation of ICE391 following exposure to a stressor by activating conjugal transfer, which requires DNA synthesis, and conceivably TLS, if the stressor is a DNA damaging agent.

Regulation of the ICE391 mutagenic SOS response can also be influenced by the rate at which SetR_{ICE391} undergoes RecA*-mediated self-cleavage. In this work, SetR_{ICE391} has been shown to undergo *in vitro* self-cleavage at both alkaline pH and in the presence of RecA*, which is common to the α -like repressors. However, the *in vitro* rate of self-cleavage of SetR_{ICE391} is limited in relation to that of LexA. Under similar conditions, the *in vitro* RecA-mediated cleavage of the *E. coli* LexA has an estimated half-life of less than 30 min [45] compared to a half-life of > 6 h for SetR_{ICE391} (Fig. 5). Excision and transfer of an ICE, along with the induction of an error-prone DNA polymerase, are events with serious consequences that require fine control and timing to favor the best evolutionary outcome. Expression of LexA-repressed genes is affected by the degree of DNA damage present, the affinity of LexA for the respective LexA box, the number of LexA boxes upstream of a gene and the efficiency at which it undergoes RecA*-mediated self-cleavage. In SXT the expression of *setCD* from the P_L promoter is subject to negative regulation by SetR_{SXT}. However, following mitomycin C induction of the SOS response, expression from P_L is not fully induced suggesting that a portion of the cellular SetR_{SXT} remains intact and functional [40]. This information, together with the data presented here, showing the reduced *in vitro* rate of self-cleavage of SetR_{ICE391}, support the idea that the rate of SetR_{ICE391} self-cleavage could have evolved to safeguard the genetic integrity of ICE391 by minimizing SOS mutagenesis and conjugal transfer, while the error-free systems act first to address any DNA damage.

Although much work remains to be done to fully understand the extent of SetR_{ICE391} activity in the mutagenic SOS response of ICE391, our findings strongly suggest that SetR_{ICE391} is a repressor of the *rumAB* operon. Mobile genetic elements such as Integrative Conjugative Elements are agents of rapid bacterial evolution. One trait of medical concern passed on by many ICEs is the acquisition of antibiotic resistance genes by horizontal gene transfer. For example, ICESXT is capable of conferring resistance to sulfamethoxazole, trimethoprim and streptomycin [62], while ICE391 integration into a host genome imparts resistance to kanamycin, as well as the heavy metal, mercury [63]. In addition, ICE391 also encodes a very potent error-prone DNA polymerase, polV_{ICE391}, which is capable of contributing to the *de novo* generation of antibiotic resistance-conferring mutations. The fact that polV_{ICE391} is so potent when sub-cloned from its native ICE391 environment [29,31,36] suggests that its mutagenic-promoting activity is normally regulated by *trans*-activating factors that are located on the intact ICE391. Here, we have identified SetR_{ICE391} as one such *trans*-activating factor, but suspect others remain to be discovered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

TLS	translesion DNA synthesis
ICE	integrating conjugative element
polV	DNA polymerase V
RecA*	activated RecA-nucleoprotein filament

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Fig. 2.

LexA and SetR regulation of polV_{ICE391}-dependent spontaneous mutagenesis. **A:** Location of the 29 open reading frames encoded by the ~21.5 kb ICE391 fragment cloned into the low-copy number plasmid, pRLH421. **B:** Arrangement of *setR*_{ICE391} and *rumAB*_{ICE391} in the low copy number plasmid, pJM1317. **C:** Spontaneous mutagenesis promoted by pRLH421 (wild-type *setR*_{ICE391}) (Red), or pJM1300 (as pRLH421, but expressing *setR*_{ICE391}(G94R) (yellow) in *lexA*⁺, *lexA*(Ind⁻) and *lexA*(Def) strains. **D:** Spontaneous mutagenesis promoted by pRLH421 (wild-type SetR_{ICE391}) (Red), or pJM1300 (as pRLH421 but expressing *setR*_{ICE391}(G94R) (yellow) in a *recA718 lexA*(Def) strain. **E:** Spontaneous mutagenesis promoted by pRW290 (*rumAB* without *setR*_{ICE391}) (Red), pJM1317 (as pRW290, but expressing wild-type *setR*_{ICE391}) (yellow), or pJM1318 (as pJM1317, but expressing *setR*_{ICE391}(G94R) (green) in *lexA*⁺, *lexA*(Ind⁻) and *lexA*(Def) strains. **F:** Spontaneous mutagenesis promoted by pRW290 (*rumAB* without *setR*) (Red), pJM1317 (wild-type *setR*_{ICE391}) (yellow), or pJM1318 (as pJM1317, but *setR*_{ICE391}(G94R) (green) in a *recA718 lexA*(Def) strain.

Fig. 3.

Specificity of SetR_{ICE391}. Mutagenesis promoted by *rumAB*, *mucAB* or *umuDC* in the presence or absence of SetR_{ICE391} were assayed in RW570 (*hisG4*(Oc) *lexA51*(Def) *umuDC596::ermGT*) harboring either pAMG1 (SetR_{ICE391}, basal level expression from Ptac) or pMal-p2X (vector) and the individually transformed compatible plasmid pRW290 (*rumAB*), pRW144 (*mucAB*) or pRW154 (*umuDC*). All three polV operons were under control of their natural LexA-regulated promoter. Fresh transformants were cultured overnight in LB and processed to measure reversion to histidine prototrophy. The results presented represent the average number of His⁺ mutants from nine plates per strain ($\pm$ standard error of the mean [SEM]).

Fig. 4.

Purification of native SetR_{ICE391}. SetR_{ICE391} was purified to greater than 95% homogeneity as described in *Materials and methods*. Each lane of the gel represents fractions taken at each step of the purification process. (U) RW1290/pDH9 in the absence of inducing treatment; (I) RW1290/pDH9 induced with 1 mM IPTG for 2 h; (Am) Soluble cell extract precipitated with 35–50% ammonium sulfate; (PC) Pooled SetR_{ICE391} fractions after elution from the phosphocellulose column; (HA) Pooled SetR_{ICE391} fractions after elution from the hydroxyapatite column. The position of SetR_{ICE391} is marked on the right of the gel and the positions of molecular weight markers (in kDa) are indicated on the left.

Fig. 5. Self-cleavage of SetR_{ICE391}. Left hand panel, RecA-mediated cleavage of SetR_{ICE391}. SetR_{ICE391} (3 µg) was incubated with activated RecA (2 µg) at 37 °C for the number of hours indicated at the top of the lanes. The products of the reaction were separated by SDS-PAGE (4–12%) and the proteins were visualized by staining with Coomassie blue. Migration positions are indicated for RecA, intact SetR_{ICE391}, and the C-terminal (SetR-C) cleavage fragment. Right hand panel, Autodigestion of SetR_{ICE391}. SetR_{ICE391} (3 µg) was incubated in 50 mM CAPS-NaOH (pH 10.0) at 37 °C for the number of hours indicated at the top of each lane. The products of the reaction were separated by SDS-PAGE (4–12%) and the proteins visualized by staining with Coomassie blue. Migration positions are indicated for intact SetR_{ICE391} and C-terminal (SetR-C) cleavage fragment.

Fig. 6.

SetR_{ICE391} binding upstream of the *rumAB* operon. **A.** Nucleotide sequence of the 123 bp DNA probe used to study binding of SetR_{ICE391} to regulatory elements upstream of *setR*_{ICE391}. **B.** Nucleotide sequence of the 194 bp DNA probe used to study binding of SetR_{ICE391} to regulatory elements upstream of *rumAB*_{ICE391}. **C.** Electrophoretic gel mobility shift assays with *E.coli* LexA and SetR_{ICE391}. The DNA probes described above were radiolabeled and incubated with increasing concentrations of either highly purified SetR_{ICE391} or LexA as specified at the bottom of each lane. DNA probe/protein complexes were incubated at 25 °C for 25 min, separated in a native 6% polyacrylamide gel and visualized on Kodak BioMax XAR film.

Fig. 7.

Identification of the SetR_{ICE391} box located upstream of *rumAB*. **A.** Nucleotide sequence of the putative SetR_{ICE391} operator and surrounding sequence. The SetR_{ICE391} operator is identified by the boxed region with areas protected during DNase I footprinting and the subsequent mutations generated within these protected areas also noted. **B.** Electrophoretic mobility shift assays with SetR_{ICE391} and both the wild-type and mutated SetR_{ICE391} operator DNA probes. The DNA probes were radiolabeled and incubated with increasing concentrations of either highly purified SetR_{ICE391} as specified at the bottom of each lane. DNA probe/protein complexes were incubated at 25 °C for 25 min, separated in a native 6% polyacrylamide gel and visualized on Kodak BioMax XAR film.

Table 1

E. coli strains and plasmids used in this study.

Strain	Relevant Genotype	Source or Reference
RW120 ^a	<i>umuDC596::cat</i>	[36]
RW518 ^a	<i>umuDC596::cat lexA3(Ind⁻)</i>	LGI stocks
RW546 ^a	<i>umuDC596::cat lexA51 (Def)</i>	[31]
RW570 ^a	<i>umuDC596::ermGTlexA51 (Def)</i>	LGI stocks
MVG114 ^a	<i>umuDC596::ermGT lexA51 (Def) recA718</i>	LGI stocks
RW1290	λ (DE3) <i>umuDC596::ermGT dinB61::ble polB::Spec recA306 srlD300::Tn10</i>	LGI stocks
Plasmid	Relevant Characteristics	Source or Reference
pRW154	Low-copy-number, Spc ^R , encoding <i>umuDC</i>	[36]
pRW144	Low-copy-number, Spc ^R , encoding <i>mucAB</i>	[36]
pRW290	Low-copy-number, Spc ^R , encoding <i>rumAB</i>	[43]
pRLH421	Low-copy-number, Spc ^R , ~21.5 kb fragment of ICE391	Genbank: U13633
pJM1300	Low-copy-number, Spc ^R , ~21.5 kb fragment of ICE391 with <i>setR</i> _{ICE391} (G94R)	This study
pJM1317	Low-copy-number, Spc ^R , encoding <i>rumAB</i> and wild-type <i>setR</i> _{ICE391}	This study
pJM1318	Low-copy-number, Spc ^R , encoding <i>rumAB</i> and <i>setR</i> _{ICE391} (G94R)	This study
pDH9	Medium-copy-number, Amp ^R , expressing <i>SetR</i> _{ICE391} from an IPTG-inducible T7 promoter	This study
pAMG1	Medium-copy-number, Amp ^R , expressing <i>SetR</i> _{ICE391} from an IPTG-inducible P _{lac} promoter	This study

^a Full genotype: *thr-1 araD139 (gpt-proA)62 lac Y1 tsx-33 gln V44 galK2 hisG4 tpsL31 xyl-5 mtl-1 argE3 thi-1 sulA211*.