Prophage Induction and Differential RecA and UmuDAB Transcriptome Regulation in the DNA Damage Responses of Acinetobacter baumannii and Acinetobacter baylyi

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Abstract

The SOS response to DNA damage that induces up to 10% of the prokaryotic genome requires RecA action to relieve LexA transcriptional repression. In Acinetobacter species, which lack LexA, the error-prone polymerase accessory UmuDAB is instead required for ddrR induction after DNA damage, suggesting it might be a LexA analog. RNA-Seq experiments defined the DNA damage transcriptome (mitomycin C-induced) of wild type, recA and umuDAb mutant strains of both A. baylyi ADP1 and A. baumannii ATCC 17978. Of the typical SOS response genes, few were differentially regulated in these species; many were repressed or absent. A striking 38.4% of all ADP1 genes, and 11.4% of all 17978 genes, were repressed under these conditions. In A. baylyi ADP1, 66 genes (2.0% of the genome), including a CRISPR/Cas system, were DNA damage-induced, and belonged to four regulons defined by differential use of recA and umuDAb. In A. baumannii ATCC 17978, however, induction of 99% of the 152 mitomycin C-induced genes depended on recA, and only 28 of these genes required umuDAb for their induction. 90% of the induced A. baumannii genes were clustered in three prophage regions, and bacteriophage particles were observed after mitomycin C treatment. These prophages encoded esv1, esvK1, and esvK2, ethanol-stimulated virulence genes previously identified in a Caenorhabditis elegans model, as well as error-prone polymerase alleles. The induction of all 17978 error-prone polymerase alleles, whether prophage-encoded or not, was recA dependent, but only these DNA polymerase V-related genes were de-repressed in the umuDAb mutant in the absence of DNA damage. These results suggest that both species possess a robust and complex DNA damage response involving both recA-dependent and recA-independent regulons, and further demonstrates that although umuDAb has a specialized role in repressing error-prone polymerases, additional regulators likely participate in these species’ transcriptional response to DNA damage.

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Introduction

Cells that experience damage to their DNA have evolved mechanisms of sensing, repairing, and replicating this damaged DNA. In most bacteria, DNA damage from various sources such as UV radiation, alkyllating chemicals (e.g., mitomycin C (MMC)), and antibiotics can induce up to 10% of the genome in this SOS response [1]. Induced SOS genes encode proteins that sense damage, control cell division, and repair, replicate and recombine DNA for continued cellular survival [2–4]. These processes are often carried out in an error-free manner, using conserved SOS DNA repair and recombination processes [3] and sulA in controlling the bacterial cell cycle [5,6]. However, DNA damage left unrepaired can also lead to the induction of SOS gene products that carry out error-prone replication of this damaged DNA. These error-prone polymerases, formed by the homodimerization of UmuC and two molecules of self-cleaving UmuD (DNA polymerase V, [7]), or DinB/DinP (DNA polymerase IV [8]) are responsible for SOS mutagenesis.

The mechanism by which these SOS genes are specifically transcribed when the cell experiences DNA damage is through relief of LexA repression [9]. This de-repression occurs after RecA binds ssDNA, an indicator of DNA damage [10], and induces LexA self-cleavage [11]. The normal state of repression in the absence of DNA damage thus prevents constitutive production of the entire SOS regulon, and SOS mutagenesis.

This general model of SOS gene induction and function, which has been developed to a significant extent in Escherichia coli [2,4], is conserved throughout proteobacterial classes, albeit imperfectly. Gamma-proteobacteria in the order Enterobacteriales often possess one LexA protein that recognizes a conserved SOS box in SOS gene promoters [2]. However, in the Pseudomonadales (containing the opportunistic, often multidrug-resistant pathogens Acinetobacter baumannii and Pseudomonas aeruginosa) and Xanthomonadiales orders, more divergent responses to DNA damage exist. For example,
*Pseudomonas putida* possesses two different LexA proteins, each controlling separate regulons [12], and *Debacter sulfurreducens* also has two LexA proteins, which do not bind the recA promoter [13]. This diversity highlights the need for additional examination of not only the mechanisms of SOS gene control but also SOS gene identity in this order.

Further components of the SOS model of gene regulation are absent in *Acinetobacter* species of this order. None of its fellow members of the family *Moraxellaceae* possess umuD homologs [14], which may have implications for the ability of these organisms to undergo SOS mutagenesis after DNA damage. Additionally, no lexA homolog has been identified in this genus [14]. Nevertheless, in the non-pathogenic genetic model organism *Acinetobacter baumannii* ADPI [15], previous investigations of the DNA damage response demonstrated that two genes are induced by mitomycin C and UV exposure in this strain. These two induced genes are recA (which unlike in other bacteria, does not require recA for its own induction [16], nor contains an SOS box in its promoter [16]), and ddrR, a gene of unknown function found only in the genus *Acinetobacter* [14]. ddrR is transcribed divergently from umuDAb [17], which is itself an unusual component of the DNA damage response of this species. UmuDAb is a UmuD homolog that is required for full induction of ddrR [17], but it is not known whether ADPI uses it to induce other genes that are, in other bacteria, part of the SOS response. UmuDAb carries out self-cleaveage in a RecA-dependent manner after cells experience diverse forms of DNA damage, and thus shares features with both the DNA polymerase V component UmuD and the LexA repressor [18]. Recent work demonstrates that in *A. baumannii* ATCC 19798, UmuDAb binds to and represses the promoters of umuDC homologs [19] and so might serve as a LexA analog for this genus.

Multiple umuD and umuC homologs co-exist in *A. baumannii* strains, and at least some of these strains (ATCC 19798, AB0057) display DNA damage-induced mutagenesis [14]. These observations suggest that these strains possess a mechanism of sensing DNA damage and inducing at least error-prone polymerase production under these conditions, and suggest that specialized UmuD function has evolved in this species. Whether this mechanism is a global response to DNA damage, induces SOS genes found in other species, or requires the action of RecA and/ or other repressors is unknown.

These unusual features in the DNA damage responses of *Acinetobacter* species prompted us to use RNAseq experiments to define the transcriptome of *A. baileyi* ADPI after DNA damage, and compare its response to that of the opportunistic pathogen, *A. baumannii* ATCC 19798. Our aims in these experiments were to determine both the existence and identity of any global DNA damage-induced transcriptome in these species, and the possible requirements for RecA and UmuDAb in regulating such a response. Although UmuDAb has been shown to regulate some DNA damage-induced genes [17,19], the limited similarity between umuDAb and lexA [14] suggests that it may not directly substitute for all LexA function, and allows for the possibility that additional regulators might exist. This stress response of this pathogen is also relevant, as environmental stresses such as dessication and exposure to UV radiation used for decontamination [20] are encountered in health care settings where nosocomial *Acinetobacter* pathogens abound. Examination of DNA damage and stress responses have been specifically identified as areas in which our knowledge of *Acinetobacter* virulence is lacking [21].

We observed that the organization, gene content, and regulation of the induced and repressed genes in the mitomycin-C-induced DNA damage transcriptome differed significantly between *A. baileyi* ADPI and *A. baumannii* ATCC 19798. These experiments also established different uses for RecA in these two species’ DNA damage responses, and suggested that UmuDAb is only one of multiple repressors of the DNA damage response in both species, serving a specialized role in regulating the transcription of error prone polymerases throughout the genome. These error-prone polymerase genes, as well as known virulence-associated genes, were found in bacteriophage particles in *A. baumannii* ATCC 19798 after DNA damage, which could facilitate the spread of mutation-inducing and other virulence genes to other bacteria.

**Materials and Methods**

**Bacterial strains and growth conditions**

*Acinetobacter* strains ADPI, ACIAD1385 (ΔrecA::KanR), and ACIAD2729 (ΔumuDAb::KanR) [22] as well as *A. baumannii* strains ATCC 19798, its isogenic recA insertion mutant [23], and a ΔumuDAb::KanR null mutant were grown at 37°C in minimal media plus succinate [17] for transcriptome and RT-qPCR analyses, and in Luria-Bertani broth for the production of phage particles. For both RNASeq transcriptome and RT-qPCR analyses, a 3 ml overnight culture, grown at 37°C at 250 rpm, was diluted 1:25 into 5 ml fresh media and grown with shaking for two hours, at which time the culture was split in two and 2 μg/ml mitomycin C (MMC) was added to one culture. Further incubation for three hours served to induce gene expression. DNA damage-induced mutagenesis after UV-C exposure was conducted as described previously [14].

**Mutant strain construction**

Null mutations of umuDAb (A1S_1389), umuD (A1S_0636) and rumB (A1S_1173) in *A. baumannii* ATCC 19798 were constructed by replacing the coding sequence of each gene with either the kanamycin resistance gene from the Invitrogen pCRTII vector (for umuDAb and umuD), or the streptomycin/spectinomycin resistance cassette from pUI1638 (for rumB), as described previously [22]. Primer sequences are listed in Table S1; “up” primers amplified DNA upstream of each coding sequence, and “down” primers amplified DNA downstream of each coding sequence. The kanamycin resistance gene was amplified with primers Kmup and Kmdw [24] and the streptomycin/spectinomycin resistance cassette was amplified from pUI1638 [25] with primers StrepSpecFor and StrepSpecRev. Splicing overlap extension PCR was used to construct linear DNA fragments from these three pieces, 300 ng of which was electroporated into *A. baumannii* ATCC 19798 cells. (In the rumB replacement, the linear fragment was first cloned into the suicide vector pEX18Gm before electroporation [26]). Transformants were selected on LB plates containing 30 μg/mL kanamycin or 10 μg/mL each of streptomycin and spectinomycin. Mutants were confirmed with PCR analyses to contain allelic replacements of the wild type allele and were not merodiploids.

**RNAseq experiments and analyses**

RNA was purified from one milliliter samples (biological triplicates for 1978; duplicates for ADPI) and processed through the Epicentre MasterPure RNA Purification kit. Further removal of contaminating DNA was performed using the Ambion DNA-free rigorous DNase treatment. RNASeq experiments were conducted with the assistance of Cofactor Genomics (St. Louis). RNA quality was assessed on a BioRad Experion instrument to have a quality corresponding to an RNA Integrity Number equal or greater than 9. Whole transcriptome RNA was extracted from total RNA by removing large and small ribosomal RNA (rRNA) using
RiboMinus Bacterial Kit (Invitrogen). Five ug of total RNA was hybridized to rRNA-specific biotin-labeled probes at 70°C for 5 minutes. The rRNA-probe complexes were then removed by streptavidin-coated magnetic beads, and tRNA free transcriptome RNA was concentrated using ethanol precipitation.

In cDNA synthesis, 1 μg of transcriptome RNA was incubated with fragmentation buffer (Illumina RNA-seq kit) for 5 minutes at 94°C. Fragmented RNA was purified with ethanol precipitation. First-strand cDNA was prepared by priming the fragmented RNA using random hexamers and followed by reverse transcription using SuperScript II (Invitrogen). The second-strand of cDNA was synthesized by incubation with second-stranded buffer, RNase Out and dNTP (Illumina RNA-seq kit) on ice for 5 minutes. The reaction mix was then treated with DNA Pol I and RNase H (Invitrogen) at 16°C for 2.5 hours.

In constructing DNA libraries, double-stranded cDNA was treated with a mix of T4 DNA polymerase, Klenow large fragment and T4 polynucleotide kinase to create blunt-ended DNA, to which a single 3’ A base was added using Klenow fragment (3’ to 5’ exonuclease) provided by an Illumina RNA-seq kit. Size selection of adaptor-ligated DNA was performed by cutting the target fragment out of a 4–12% acrylamide gel. The amplified DNA library was obtained by in-gel PCR using a Phusion High-Fidelity system (New England Biolabs).

Sequencing and cluster generation was performed according to the sequencing and cluster generation manuals from Illumina (Cluster Station User Guide and Genome Analyzer Operations Guide). Primary data were generated using the Illumina Pipeline version SCS 2.8.0 paired with QLB 1.3.0. NovaAlign version 2.07.05 was used for all sequence alignment; aligner algorithms can be obtained from novocraft.com. The coverage depth for sequencing of the A. baylyi ADP1 libraries was an average of 67.5-fold for the wild type strain, 69.7-fold for ACIAD1385, and 148-fold for ACIAD2729, with an average percent coverage of reference bases of 95.8%, 98.2%, and 99.8%, respectively. Coverage depth for the sequenced A. baumannii ATCC 17978 libraries was 235-fold for the wild type strain (99.3% of reference bases covered), 238-fold for the recA strain (98.7% of reference bases covered), and 203-fold for the umuDAb strain (97.3% of reference bases covered). An average of 878 million to 1.1 billion total bases were generated for each A. baumannii ATCC 17978 library and over 300 million total bases were generated for each A. baileyi ADP1 library. Clusters were linearly normalized by multiplying each sample’s coverage by the total reads of the lower read-count sample divided by the respective sample’s total reads, and the induction ratio of reads between MMC-treated and untreated samples was then calculated. Genes were considered induced if this ratio was greater than or equal to 2.0, and untreated samples was then calculated. Genes were considered induced if this ratio was greater than or equal to 2.0, and untreated samples was then calculated. Genes were considered induced if this ratio was greater than or equal to 2.0, and untreated samples was then calculated. Genes were considered induced if this ratio was greater than or equal to 2.0, and untreated samples was then calculated. Genes were considered induced if this ratio was greater than or equal to 2.0, and untreated samples was then calculated.

RT-qPCR analysis

RNA samples for RT-qPCR were produced from 1 mL of triplicate biological samples with the Epicentre MasterPure RNA Purification Kit, after which additional removal of DNA was performed using Ambion DNA-free rigorous DNAseI treatment. Removal of contaminating DNA was verified by the absence of PCR products amplified when PCR was performed with primers listed in Table S2 and S3: 17978umuDCRev1For and 17978umuDCRev2For for A. baumannii strains 17978, 17978 recA, and 17978 ΔumuDB. For A. baileyi strains ADP1 and ACIAD1385, umuDAb#2RTFor and umuDAb#2RTRev were used, and for ACIAD2729, dnaNRTFor and dnaNRTRRev were used. Genomic DNA was used as a positive control. RNA sample quality was confirmed on an E-Gel EX 2% agarose gel (Invitrogen) before use.

cDNA was synthesized from 1 μg of RNA with oligo(dT) and random hexamers by a modified Moloney murine leukemia virus reverse transcriptase in a 25 μL reaction (Bio-Rad iScript cDNA Synthesis kit). Five μL of a 1:100 dilution of this cDNA was used to perform RT-qPCR using BioRad iTaq SYBR Green Supermix on an Applied Biosystems 7300 Real-Time PCR system in a 15 μL reaction. Technical triplicates were run for each of the three biological replicates in ABI MicroAmp Optical 96-well clear reaction plates, with the following cycling conditions: 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A dissociation step of 95°C for 15 seconds, followed by 60°C for 30 seconds and 95°C for 15 seconds was used to check product integrity. No template controls for each primer set confirmed absence of product formation. Each RT-qPCR plate contained wild type and one mutant strain sample, comparing reference primers and test primers for the gene of interest. RT-qPCR primers were designed using PrimerBlast (NCBI) and are listed in Tables S2 and S3. PCR efficiency was evaluated for each primer set by dilution of genomic DNA over 5 logs of template concentration and was between 94% and 105% for all primer sets. Efficiency was calculated using the formula E = 10^{(-1/ΔC})_{\text{R}}^\text{target} and the standard curve generated with the primer set, where efficiency = (E-1)×100%. Primer concentration used was 400 μM. All gene primer sets were compared to the reference gene primer set 16SrRNA#RTFor and 16SrRNA#2RTRev for A. baileyi ADP1 (Table S2) and 1797816sRNRARTFor and 1797816sRNRARTRev for A. baumannii ATCC 17978 (Table S3). Validation of these reference primers was performed by observing no significant difference between MMC-treated and untreated samples in either A. baumannii ATCC 17978 or A. baileyi ADP1 in six independent experiments (p>0.05 in a paired t-test). Transcriptional changes were calculated using the 2^(-ΔΔC) method [29] and GraphPad InStat was used to conduct all statistical analyses.

Bacteriophage purification, electron microscopy and analyses

Phages were produced in cultures grown in LB broth. Overnight cultures were diluted 1:25 into fresh medium and grown at 37°C with shaking for 1 hour before MMC at 2 μg/mL was added. The cultures’ optical density at 600 nm was measured each hour for six additional hours after induction with MMC. At either three or six hours, 1 mL of culture was centrifuged at 13,000 rpm for two minutes, and the supernatant was filtered through a 0.22 μm filter. This filtrate was centrifuged at 13,200 rpm for one hour at room temperature and the pellet was resuspended in phage buffer (10 mM Tris, pH 7.5, 10 mM MgSO4, 68.5 mM NaCl, 1 mM CaCl2). These samples were processed through the Ambion DNA-free DNase Treatment & Removal kit if PCR analyses were performed.

The resulting phage suspension was processed for transmission electron microscopy. Phage samples were placed on freshly made carbon coated formvar grids for 5 minutes, rinsed with phage buffer and deionized water for ten seconds each, and stained twice with 1% uranyl acetate for one minute each. Uranyl acetate was wicked off and the grid was air dried. Micrographs were taken.
using 80kV accelerating voltage on a JEOL 100CX transmission electron microscopy onto Kodak 4459 film, then scanned with a Minolta Damage Scan Multi Pro film scanner at 2400 dpi. The capsid diameter, tail width, and tail length of twelve phage particles observed in micrographs was measured with Image J software [30]. The arithmetic mean of the measurements was reported. Analysis of the genome structure and content of the three cryptic prophage regions was performed using the web server Phage Search Tool (PHAST) [51].

Results

Previous reports had indicated that ddrR [17] and recA [16] were induced by DNA damage in A. baileyi ADP1, and recent observations indicated that multiple error prone polymerases were induced by various forms of DNA damage in A. baumannii ATCC 17978 [19,32]. However, in the absence of a LexA homolog encoded by these species [14], it was not known whether multiple genes were induced in these species, nor how this response might be regulated. RNA-Seq experiments were performed to test whether A. baileyi ADP1 and A. baumannii ATCC 17978 (henceforth abbreviated as ADP1 and 17978, respectively) possessed a genome-wide transcriptional response to mitomycin C exposure. Genes were considered induced (or repressed) if their expression increased (or decreased) by 2.0-fold or more, relative to their expression in untreated cultures.

A. baileyi ADP1 possess a DNA damage transcriptomes of SOS response genes, a CRISPR/Cas system, and other genes

Sixty-six genes, or 2.0%, of all ADP1 genes were induced (Table 1), indicating a global system of regulating gene expression in response to this form of DNA damage. These 66 induced genes were widely dispersed throughout the chromosome, and included 8 putative operons of two genes each. In addition to these induced genes, an astonishing 38.4% of all ADP1 genes were repressed upon DNA damage.

A core set of 6 SOS genes for gamma proteobacteria such as Acinetobacter includes recA, sbb, rcaA, rucE, recN, and wcaI [33], with a larger set of 36 genes induced in Escherichia coli [2] and Pseudomonas aeruginosa [34], the best-studied organism in the order to which A. baumannii belongs (Pseudomonadales). Surprisingly, only 6 of these 36 genes were induced, and 7 genes were repressed, while 9 genes were neither induced nor repressed (Table 2). Only 4 of these 36 genes were induced, with core SOS genes recA, umuD and sbb induced, similar to ADP1. Two genes were repressed (hodB and rusC, also repressed in ADP1), while 16 genes were neither induced nor repressed, and the same 14 genes as were absent from the ADP1 genome were absent in the 17978 genome. There was no significant difference between ADP1 and 17978 in the number of genes in these four classes (induced, repressed, unaffect, absent) as tested with Chi-square analysis (p>0.05).

In contrast to the dispersal of induced genes throughout the chromosome in ADP1, the location of 90% of all 17978 induced genes nearly perfectly overlapped with three regions predicted to contain prophages by our analysis with the Phage Search Tool (PHAST), a web server designed to rapidly and accurately identify, analyze, and annotate prophages [31] (Figure 1B). These three regions were also the only regions to be identified as cryptic prophages (CP, CP5, CP9, and CP14) in 17978, based on their presence in some but not all epidemic-associated A. baumannii strains [38]. Ninety-nine percent of all genes within these prophage regions were induced.

The DNA damage transcriptome of A. baumannii ATCC 17978 includes three prophages

In A. baumannii ATCC 17978, 152 genes, or 3.7% of the genome, was induced (Table 3), indicating that the expression of multiple genes was regulated in response to this form of DNA damage. In 17978, as for ADP1, few of the canonical SOS genes responded to MMC-induced DNA damage as in the E. coli model (Table 2). 4 of these 36 genes were induced, with core SOS genes recA, umuD and sbb induced, similar to ADP1. Two genes were repressed (hodB and rusC, also repressed in ADP1), while 16 genes were neither induced nor repressed, and the same 14 genes as were absent from the ADP1 genome were absent in the 17978 genome. This prophage may have been horizontally acquired from another A. baumannii strain [15].

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| Gene identity | Gene name | Function | Regulation | Fold Induction |
|---------------|-----------|----------|------------|---------------|
| ACIAD1385     | recA      | DNA recombination and repair | NA*        | 5.2           |
| ACIAD0445     | gst       | Glutathione S-transferase (detoxification) | Neither    | 190.0         |
| ACIAD2480     | Conserved hypothetical protein | Neither | 8.5        |
| ACIAD2482     | csy3      | RAMP superfamily protein/Cas system | Neither    | 8.5           |
| ACIAD2483     | cas6      | Endoribonuclease involved in crRNA biogenesis | Neither    | 8.3           |
| ACIAD0446     | Conserved hypothetical protein | Neither | 7.9        |
| ACIAD3449     | ssb       | RecBCD nuclease ssDNA-binding protein | Neither    | 7.4           |
| ACIAD0724     | nrdA      | Ribonucleoside diphosphate reductase, alpha subunit | Neither    | 7.0           |
| ACIAD2481     | csy2      | RAMP superfamily protein/Cas system | Neither    | 6.5           |
| ACIAD0722     | nrdB      | Ribonucleoside-diphosphate reductase, beta subunit | Neither    | 5.4           |
| ACIAD0005     | Conserved hypothetical protein | Neither | 5.3        |
| ACIAD3565     | Conserved hypothetical protein | Neither | 4.6        |
| ACIAD3649     | Conserved hypothetical protein | Neither | 3.9        |
| ACIAD2210     | rpmE      | 50S ribosomal protein L31 | Neither    | 3.6           |
| ACIAD3535     | raiA      | Stress response ribosomal inhibitor | Neither    | 3.0           |
| ACIAD1205     | dps       | Stress response DNA-binding protein, starvation induced resistance to H2O2, ferritin-like | Neither    | 2.8           |
| ACIAD2479     | Conserved hypothetical protein | Neither | 2.8        |
| ACIAD3545     | Putative esterase | Neither | 2.5        |
| ACIAD2295     | Putative oxidoreductase | Neither | 2.5        |
| ACIAD3566     | Hypothetical protein | Neither | 2.5        |
| ACIAD2652     | gyrA      | DNA gyrase, subunit A, type II topoisomerase | Neither    | 2.4           |
| ACIAD0151     | guaA      | Glutamine aminotransferase | Neither    | 2.3           |
| ACIAD3503     | guaB      | IMP dehydrogenase | Neither    | 2.2           |
| ACIAD0010     | Putative Fe/S cluster chaperone | Neither | 2.2        |
| ACIAD0868     | Conserved hypothetical protein | Neither | 2.1        |
| ACIAD1473     | Conserved hypothetical protein | recA | 5.2         |
| ACIAD2484     | cas1      | DNAase | recA | 3.7         |
| ACIAD1772     | Conserved hypothetical protein | recA | 3.7         |
| ACIAD3455     | uvrA      | Excinuclease ABC subunit A | recA | 3.4         |
| ACIAD2614     | ruvA      | Holliday junction helicase subunit A | recA | 2.4         |
| ACIAD0002     | dnaN      | DNA polymerase III, beta chain | recA | 2.2         |
| ACIAD2613     | dgt       | dGTPase | recA | 2.1         |
| ACIAD3408     | Endonuclease G | recA | 2.1         |
| ACIAD2729     | umuDAb    | Component of DNA polymerase V | recA** | 4.0         |
| ACIAD2730     | ddrA      | DNA damage-inducible protein | recA and umuDAb | 26.2       |
| ACIAD1478     | hemO      | Heme oxygenase | recA and umuDAb | 3.2       |
| ACIAD1474     | Conserved hypothetical protein | recA and umuDAb | 2.5       |
| ACIAD0334     | fadA      | 3-ketoacyl-CoA thiolase | recA and umuDAb | 2.4       |
| ACIAD0335     | fadB      | Fatty oxidation complex alpha subunit | recA and umuDAb | 2.3       |
| ACIAD2034     | Putative signal peptide protein | recA and umuDAb | 2.2       |
| ACIAD0387     | acuA      | Fimbrial-like protein | recA and umuDAb | 2.2       |
| ACIAD1208     | Fatty acid desaturase | recA and umuDAb | 2.1       |
| ACIAD2103     | ahpC      | alkyl hydroperoxide reductase (detoxification) | recA and umuDAb | 2.1       |
| ACIAD1024     | Conserved hypothetical protein | recA and umuDAb | 2.1       |
| ACIAD0697     | ompA-like | recA and umuDAb | 2.0       |
| ACIAD0006     | Hypothetical protein | umuDAb | 4.2       |
| ACIAD0401     | rpsO      | 30S ribosomal protein S15 | umuDAb | 2.6       |
| ACIAD3325     | rpoZ      | RNA polymerase, omega subunit | umuDAb | 2.3       |
Virulence-associated genes were also induced in 17978. Previous studies in a Caenorhabditis elegans model of A. baumannii ATCC 17978 infection demonstrated that ethanol-stimulation of virulence was dependent upon 12 esv genes [27]. Two of these, esvK1 and esvK2, which were encoded in CP14, were induced, with the induction of esvK1 further tested and confirmed in RT-qPCR experiments. While the induction of esvI, encoded by CP9, fell just below the RNASeq induced cutoff ratio of 2.0, it was induced ∼5-fold after MMC treatment in RT-qPCR experiments. No

Table 1. Cont.

| Gene identity | Gene name | Function | Regulation | Fold Induction |
|---------------|-----------|----------|------------|---------------|
| ACIAD3602     | Conserved hypothetical protein | umuDAb  | 2.3        |
| ACIAD3402     | iscA      | Iron-binding protein | umuDAb  | 2.3 |
| ACIAD3506     | aceF      | Dihydrolipoamide S-acetyltransferase | umuDAb  | 2.2 |
| ACIAD3809     | Lipase    | umuDAb  | 2.2        |
| ACIAD3156     | htpG      | Heat shock protein; chaperone Hsp90 | umuDAb  | 2.1 |
| ACIAD3654     | dnaK      | Heat shock protein; chaperone Hsp70 | umuDAb  | 2.1 |
| ACIAD7072     | Conserved hypothetical protein | umuDAb  | 2.1        |
| ACIAD3564     | Conserved hypothetical protein | umuDAb  | 2.1        |
| ACIAD3155     | mdh       | Malate dehydrogenase | umuDAb  | 2.0 |
| ACIAD0042     | Acyl carrier | umuDAb  | 2.0        |
| ACIAD3604     | Putative histidine triad | umuDAb  | 2.0        |
| ACIAD2938     | rpmA      | 50S ribosomal protein L27 | umuDAb  | 2.0 |
| ACIAD3330     | bfrB      | Bacterioferritin | umuDAb  | 2.0 |
| ACIAD3370     | Conserved hypothetical protein | umuDAb  | 2.0        |

* recA expression could not be evaluated in the null recA mutant, but was not regulated by umuDAb.
** umuDAb expression could not be evaluated in the null umuDAb mutant, but was regulated by recA.

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Table 2. Regulation and presence of canonical SOS genes in Acinetobacter species.

| Gene | A. baylyi ADP1 | A. baumannii 17978 |
|------|----------------|-------------------|
| recA | Induced        | Induced           |
| ssb  | Induced        | Induced           |
| umuDAb | Induced    | Induced           |
| dnaN | Induced        | No Change         |
| ruvA | Induced        | No Change         |
| uvrA | Induced        | No Change         |
| umuD (0636*) | Not in genome | Induced           |
| umuC (0637) | Not in genome | Induced           |
| umuD (1174) | Not in genome | Induced           |
| rnuB (1173) | Not in genome | Induced           |
| umuc (2015) | Not in genome | Induced           |
| holB | Repressed      | Repressed         |
| ruvC | Repressed      | Repressed         |
| uvrC | Repressed      | No Change         |
| dinB/dinP | Repressed   | No Change         |
| recN | Repressed      | No Change         |
| recG | Repressed      | No Change         |
| dnaQ | Repressed      | No Change         |
| frxK, gyrB, hupB, polA, recF, ruvB, uvrB, uvrD, uvrE, yebQ, yigN | No Change | No Change |
| dinI, dinG, hokE, lexA, molR, pcsA, polB, sbmC, sulA, ybfE, ydjM, ydicQ, yebG, yigN | Not encoded in genome | Not encoded in genome |

* Four digit numbers correspond to A15 gene numbers in A. baumannii.

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CRISPR-Cas system is present in 17978, although some isolates of the EU clone lineage I possess a CRISPR-Cas system [40].

Although none of the genes on the 17978 plasmids pAB1 and pAB2 were induced, 6 of 11 pAB1 genes, and 2 of 6 pAB2 genes were repressed. Overall, 11.4% of all 17978 genes were repressed.

The A. baylyi DNA damage transcriptome includes four different regulons of MMC-induced genes

In the SOS response of gammaproteobacteria, RecA action is typically required to relieve SOS genes from repression by either LexA [4] or a prophage repressor [41]. We conducted RNASeq analysis on both recA and umuDAb mutant strains of ADP1 and 17978 to test whether recA regulated these transcriptomes, and whether umuDAb was a global regulator of DNA damage-induced (and/or repressed) genes in a LexA-analogous manner.

These experiments demonstrated a complex picture of regulation, with the ADP1 transcriptome possessing four regulons of induced genes that differentially required umuDAb and recA (Table 1). Figure 2A shows these four regulons, which were supported by statistical testing (repeated measures analysis of variance within each regulon; p<0.05). Twelve genes were regulated by both umuDAb and recA; 13 genes required recA only. Unexpectedly, we found a regulon of 22 genes that were induced after DNA damage but required neither recA nor umuDAb for this induction. Additionally, 17 genes were regulated only by umuDAb, but all of these were still moderately induced in the umuDAb mutant, having an average induction ratio of 1.70-fold (only slightly below the cutoff for being considered induced), and were not investigated further. These categories were validated by RT-qPCR experiments: dhhR required both recA and umuDAb, dnaN required only recA, and sbb and rulA required neither recA nor umuDAb. In the eight induced operons, the regulation was the same throughout the operon, supporting the categorization and physiological relevance of the regulation method.

This variety in regulatory requirements also extended to the induced SOS genes (Figure 3A). None of the five canonical SOS genes that were induced (recA, dnaN, uvrA, ssb, and ruvA) depended upon umuDAb. Only three of the five SOS genes were recA-regulated, none of the five were umuDAb-regulated, and strikingly, ssb was regulated by neither recA nor umuDAb (Figure 3A). This regulation was confirmed in RT-qPCR experiments.

In contrast, throughout the ADP1 genome, including all repressed SOS genes, 87% of the repressed genes required only umuDAb to be repressed, with just 6% requiring both umuDAb and recA, and 7% requiring neither of these genes for repression (Figure 2B).

The A. baumannii DNA damage transcriptome requires RecA regulation and displays a specialized regulatory role for the UmuDAb repressor

In contrast to ADP1, 17978 exhibited only a recA-dependent path of inducing genes—with the exception of recA itself and A1S_2020, which were induced 2.0 to 2.2-fold, respectively, in the recA mutant. However, the 17978 induced transcriptome
Table 3. Genes induced in A. baumannii 17978 after MMC-induced DNA damage and their regulation by UmuDAb and RecA.

| Gene identity | Gene name | Function | Regulation | Fold Induction |
|---------------|-----------|----------|------------|----------------|
| A1S_1962      | recA      | DNA strand exchange and recombination* | Neither | 5.6            |
| A1S_0408      | gst       | Glutathione S-transferase* (detoxification) | recA | 73.5           |
| A1S_2026      | Hypothetical protein | | recA | 30.8           |
| A1S_3617      | Hypothetical protein | | recA | 23.0           |
| A1S_3764      | Hypothetical protein | | recA | 20.4           |
| A1S_3618      | Hypothetical protein | | recA | 18.0           |
| A1S_3779      | Hypothetical protein | | recA | 17.3           |
| A1S_1159      | Hypothetical protein | | recA | 17.0           |
| A1S_3765      | Hypothetical protein | | recA | 16.8           |
| A1S_3762      | Hypothetical protein | | recA | 15.2           |
| A1S_2027      | Hypothetical protein | | recA | 14.7           |
| A1S_1145      | Putative Cro protein | | recA | 14.5           |
| A1S_1157      | Hypothetical protein | | recA | 14.2           |
| A1S_2021      | Hypothetical protein | | recA | 13.6           |
| A1S_1156      | Hypothetical protein | | recA | 13.5           |
| A1S_2024      | Glutamate 5-kinase | | recA | 13.4           |
| A1S_1161      | Hypothetical protein | | recA | 12.6           |
| A1S_1158      | Putative signal peptide | | recA | 12.5           |
| A1S_1162      | Hypothetical protein | | recA | 12.0           |
| A1S_1163      | Hypothetical protein | | recA | 11.7           |
| A1S_3614      | Hypothetical protein | | recA | 11.4           |
| A1S_1160      | Hypothetical protein | | recA | 11.3           |
| A1S_2022      | Putative tail fiber | | recA | 10.9           |
| A1S_2031      | Hypothetical protein | | recA | 10.8           |
| A1S_3608      | Hypothetical protein | | recA | 10.7           |
| A1S_3615      | Hypothetical protein | | recA | 10.5           |
| A1S_3766      | Hypothetical protein | | recA | 10.0           |
| A1S_3763      | Hypothetical protein | | recA | 9.8            |
| A1S_3760      | Hypothetical protein | | recA | 9.2            |
| A1S_3772      | Hypothetical protein | | recA | 9.2            |
| A1S_2018      | Tail tape measure protein | | recA | 9.2            |
| A1S_2029      | Hypothetical protein | | recA | 9.2            |
| A1S_3611      | Hypothetical protein | | recA | 9.1            |
| A1S_1147      | DNA methylase | | recA | 9.1            |
| A1S_3761      | Hypothetical protein | | recA | 9.1            |
| A1S_1155      | Putative phage-related protein | | recA | 8.9            |
| A1S_3613      | Hypothetical protein | | recA | 8.9            |
| A1S_3754      | Hypothetical protein | | recA | 8.9            |
| A1S_1146      | Site-specific methylase | | recA | 8.8            |
| A1S_3606      | Hypothetical protein | | recA | 8.7            |
| A1S_3607      | Hypothetical protein | | recA | 8.7            |
| A1S_3758      | Hypothetical protein | | recA | 8.7            |
| A1S_3700      | Hypothetical protein | | recA | 8.6            |
| A1S_3757      | Hypothetical protein | | recA | 8.5            |
| A1S_2023      | Hypothetical protein | | recA | 8.5            |
| A1S_1166      | Hypothetical protein | | recA | 8.4            |
| A1S_3612      | Hypothetical protein | | recA | 8.3            |
| A1S_1149      | Hypothetical protein | | recA | 8.2            |
| A1S_2019      | Hypothetical protein | | recA | 8.1            |
| A1S_3771      | Hypothetical protein | | recA | 8.0            |
| Gene identity | Gene name          | Function                              | Regulation | Fold Induction |
|---------------|--------------------|---------------------------------------|------------|---------------|
| A15_2016      | Phage-related lysozyme | recA                                  | 8.0        |               |
| A15_3616      | Hypothetical protein  | recA                                  | 7.9        |               |
| A15_3609      | Hypothetical protein  | recA                                  | 7.8        |               |
| A15_3776      | Hypothetical protein  | recA                                  | 7.8        |               |
| A15_3773      | Hypothetical protein  | recA                                  | 7.8        |               |
| A15_1153      | Putative phage-related protein | recA | 7.7 |               |
| A15_1595      | Hypothetical protein  | recA                                  | 7.7        |               |
| A15_2017      | Hypothetical protein  | recA                                  | 7.7        |               |
| A15_3767      | Hypothetical protein  | recA                                  | 7.6        |               |
| A15_1148      | Hypothetical protein  | recA                                  | 7.5        |               |
| A15_2036      | DNA cytosine methyltransferase | recA | 7.5 |               |
| A15_1152      | Putative helicase     | recA                                  | 7.3        |               |
| A15_1154      | Putative bacteriophage protein | recA | 7.3 |               |
| A15_2033      | Hypothetical protein  | recA                                  | 7.3        |               |
| A15_1167      | Hypothetical protein  | recA                                  | 7.1        |               |
| A15_1169      | Hypothetical protein  | recA                                  | 7.1        |               |
| A15_2039      | Phage nucleotide binding protein | recA | 7.1 |               |
| A15_1586      | esvK1 Ethanol-stimulated virulence protein** | recA | 7.1 |               |
| A15_1594      | Hypothetical protein  | recA                                  | 7.1        |               |
| A15_1164      | Putative phage tail tape measure protein | recA | 7.0 |               |
| A15_3620      | Hypothetical protein  | recA                                  | 7.0        |               |
| A15_3755      | Holin                 | recA                                  | 7.0        |               |
| A15_1151      | Hypothetical protein  | recA                                  | 6.7        |               |
| A15_1165      | Putative phage tail tape measure protein | recA | 6.7 |               |
| A15_3605      | Hypothetical protein  | recA                                  | 6.7        |               |
| A15_2030      | Putative phage associated protein | recA | 6.7 |               |
| A15_1150      | Hypothetical protein  | recA                                  | 6.6        |               |
| A15_1168      | Hypothetical protein  | recA                                  | 6.5        |               |
| A15_3610      | Hypothetical protein  | recA                                  | 6.2        |               |
| A15_3778      | Hypothetical protein  | recA                                  | 6.2        |               |
| A15_3756      | Hypothetical protein  | recA                                  | 6.1        |               |
| A15_2032      | Hypothetical protein  | recA                                  | 6.0        |               |
| A15_1171      | Hypothetical protein  | recA                                  | 5.8        |               |
| A15_3777      | Hypothetical protein  | recA                                  | 5.8        |               |
| A15_3768      | Hypothetical protein  | recA                                  | 5.8        |               |
| A15_3678      | Hypothetical protein* | recA | 5.8 |               |
| A15_1170      | Hypothetical protein  | recA                                  | 5.7        |               |
| A15_3603      | Hypothetical protein* | recA | 5.7 |               |
| A15_3604      | Hypothetical protein  | recA                                  | 5.7        |               |
| A15_3621      | Hypothetical protein  | recA                                  | 5.7        |               |
| A15_3770      | Hypothetical protein  | recA                                  | 5.6        |               |
| A15_1591      | Major capsid protein  | recA                                  | 5.6        |               |
| A15_3696      | Hypothetical protein  | recA                                  | 5.6        |               |
| A15_3702      | Hypothetical protein  | recA                                  | 5.3        |               |
| A15_2035      | Hypothetical protein  | recA                                  | 5.3        |               |
| A15_3699      | Hypothetical protein  | recA                                  | 5.1        |               |
| A15_3769      | Hypothetical protein  | recA                                  | 5.0        |               |
| A15_3693      | Hypothetical protein  | recA                                  | 4.9        |               |
| A15_3287      | ssb                   | RecBCD nuclease ssDNA-binding protein* | recA | 4.9 |               |
| Gene identity | Gene name | Function | Regulation | Fold Induction |
|---------------|-----------|----------|------------|----------------|
| A1S_1143      | Hypothetical protein | recA | 4.7 |
| A1S_3705      | Hypothetical protein | recA | 4.7 |
| A1S_1175      | Phage integrase | recA | 4.6 |
| A1S_3759      | Hypothetical protein | recA | 4.4 |
| A1S_3695      | Hypothetical protein | recA | 4.4 |
| A1S_1172      | Putative transposase | recA | 4.3 |
| A1S_3694      | Hypothetical protein | recA | 4.3 |
| A1S_2038      | Hypothetical protein | recA | 4.0 |
| A1S_1192      | Putative Phage head-tail adaptor | recA | 4.0 |
| A1S_1156      | Hypothetical protein | recA | 4.0 |
| A1S_1599      | Hypothetical protein | recA | 3.7 |
| A1S_3682      | Hypothetical protein* | recA | 3.6 |
| A1S_1593      | Hypothetical protein | recA | 3.5 |
| A1S_1597      | Phage tail tape measure protein | recA | 3.5 |
| A1S_1595      | Hypothetical protein | recA | 3.1 |
| A1S_3697      | Hypothetical protein | recA | 3.1 |
| A1S_3701      | Hypothetical protein | recA | 3.0 |
| A1S_1587      | esvK2 | Terminase; Ethanol-stimulated virulence protein** | recA | 2.9 |
| A1S_1589      | Hypothetical protein | recA | 2.9 |
| A1S_1590      | Peptidase U35 phage prohead HK97 | recA | 2.9 |
| A1S_3804      | Hypothetical protein* | recA | 2.9 |
| A1S_1600      | Lysozyme | recA | 2.6 |
| A1S_3698      | Hypothetical protein | recA | 2.6 |
| A1S_3727      | Hypothetical protein* | recA | 2.1 |
| A1S_2025      | Hypothetical protein | recA and umuDAb | 19.1 |
| A1S_1388      | ddrR | DNA damage-inducible protein* | recA and umuDAb | 13.7 |
| A1S_2028      | Phage putative head morphogenesis protein | recA and umuDAb | 10.1 |
| A1S_1174      | umuD | DNA polymerase V component | recA and umuDAb | 9.6 |
| A1S_0636      | umuD | DNA polymerase V component* | recA and umuDAb | 8.0 |
| A1S_3767      | Hypothetical protein | recA and umuDAb | 7.6 |
| A1S_1173      | rumB | DNA-directed DNA polymerase | recA and umuDAb | 5.8 |
| A1S_3619      | Hypothetical protein | recA and umuDAb | 5.4 |
| A1S_3622      | Hypothetical protein | recA and umuDAb | 5.1 |
| A1S_1144      | Repressor, S24 family peptidase | recA and umuDAb | 4.9 |
| A1S_3759      | Hypothetical protein | recA and umuDAb | 4.4 |
| A1S_2015      | umuC | DNA-directed DNA polymerase | recA and umuDAb | 3.8 |
| A1S_1389      | umuDAb | DNA polymerase V component* | recA and umuDAb | 3.8 |
| A1S_3704      | Hypothetical protein | recA and umuDAb | 3.3 |
| A1S_1598      | Hypothetical protein | recA and umuDAb | 3.1 |
| A1S_2040      | Putative phage integrase | recA and umuDAb | 3.0 |
| A1S_3701      | Hypothetical protein | recA and umuDAb | 3.0 |
| A1S_0637      | umuC | DNA-directed DNA polymerase* | recA and umuDAb | 2.8 |
| A1S_1600      | Lysozyme | recA and umuDAb | 2.6 |
| A1S_0278      | tmT | Threonine tRNA* | recA and umuDAb | 2.5 |
| A1S_2014      | SOS response associated thiol autopeptidase | recA and umuDAb | 2.2 |
| A1S_3774      | Hypothetical protein | recA and umuDAb | 2.2 |
| A1S_3775      | Hypothetical protein | recA and umuDAb | 2.2 |
| A1S_2236      | tmW | Tryptophan tRNA* | recA and umuDAb | 2.2 |
Table 3. Cont.

| Gene identity | Gene name       | Function                          | Regulation     | Fold Induction |
|---------------|-----------------|-----------------------------------|----------------|---------------|
| A15_2034      | Hypothetical protein | recA and umuDAb                  | 2.1            |
| A15_3706      | Hypothetical protein | recA and umuDAb                  | 2.1            |
| A15_0421      | Protein chain initiation factor IF-1* | recA and umuDAb             | 2.1            |
| A15_2020      | Hypothetical protein | umuDAb                           | 7.0            |

*Indicates that the induced gene is not part of a prophage region.
** See reference [27].

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We further tested whether all of the prophage-encoded error-prone polymerase alleles (CP5 (A1S_1173/1174, umuDrumB) and CP9 (A1S_2014-15)) were regulated similarly to their chromosomal counterparts umuDC (A1S_0636-0637) and the regulatory umuDCB gene (A1S_1389). All were regulated by recA and de-repressed in the umuDCB mutant (i.e. had high expression in the absence of MMC exposure), with this regulation confirmed by RT-qPCR experiments (Figure 4). This was not observed for non-umuDC-related alleles, either prophage- or chromosomally-encoded (recA, exk1, and ssb); although recA-dependent, they were not regulated by umuDAb or de-repressed in the umuDCB mutant (Figure 4).

DNA damage in A. baumannii induces bacteriophage particle production that contain virulence genes

PHAST characterization of the prophages apparently encoded by the three induced regions of the 17978 chromosome indicated that the majority of each prophage’s genes (65% in CP14, 70% in CP5, and 81% in CP9) were either conserved hypothetical phage genes or phage genes of a function identified by homology (Figure 1B, Table 4). Of these genes typically found in bacteriophages, 65 - 78% (depending on the CP) were most similar to phage genes from the viral family Siphoviridae. In CP9, 68% of the phage genes were most similar (identity ranging from contained two DNA-damage induced regulons: i) 123 genes regulated by recA (i.e. umuDAb-independent), and ii) 27 genes regulated by recA and umuDAb (i.e. umuDAb-dependent) (Table 3, Figure 2A). Within the umuDAb-independent regulon, there was a significant difference between the induction of the wild type vs. the umuDAb samples (p<0.05 in a Wilcoxon matched-pairs signed-ranks test), suggesting a possibly different role of umuDAb from simple repression. Consistent with the proportions of genes in these two regulons, 85% of the induced genes in the three prophages CP5, CP9, and CP14 required recA only, and this regulation was not significantly different for conserved hypothetical genes vs. genes typically found in bacteriophages (p>0.05, Fisher’s exact test.). These observations were consistent with the possibility of gene repression by a prophage-encoded repressor. Of the 8 induced canonical SOS genes (which includes 6 alleles of umuDC), only the umuDC alleles were dependent on umuDAb for induction (Figure 3A). The recA and ssb genes’ induction were umuDAb independent (Figure 3A). This regulation of ssb, umuDAb, and recA was confirmed in RT-qPCR experiments.

In the DNA damage-repressed transcriptome, this pattern was reversed: umuDAb was required for 99% of the genes’ repression, with recA also required in ~49% of the cases. This was also observed in the repressed SOS genes, where umuDAb was required for repression after DNA damage of holB and recC, but recA was required for repression as well (Figure 3B). However, repression of 7 of the 8 genes located on the plasmids pAB1 and pAB2 required both umuDAb and recA.

We further tested whether all of the prophage-encoded error-prone polymerase alleles (CP5 (A1S_1173/1174, umuDrumB) and CP9 (A1S_2014-15)) were regulated similarly to their chromosomal counterparts umuDC (A1S_0636-0637) and the regulatory umuDCB gene (A1S_1389). All were regulated by recA and de-repressed in the umuDCB mutant (i.e. had high expression in the absence of MMC exposure), with this regulation confirmed by RT-qPCR experiments (Figure 4). This was not observed for non-umuDC-related alleles, either prophage- or chromosomally-encoded (recA, exk1, and ssb); although recA-dependent, they were not regulated by umuDAb or de-repressed in the umuDCB mutant (Figure 4).

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| Gene identity | Gene name       | Function                          | Regulation     | Fold Induction |
|---------------|-----------------|-----------------------------------|----------------|---------------|
| A15_2034      | Hypothetical protein | recA and umuDAb                  | 2.1            |
| A15_3706      | Hypothetical protein | recA and umuDAb                  | 2.1            |
| A15_0421      | Protein chain initiation factor IF-1* | recA and umuDAb             | 2.1            |
| A15_2020      | Hypothetical protein | umuDAb                           | 7.0            |

*Indicates that the induced gene is not part of a prophage region.
** See reference [27].

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Figure 2. Distribution of regulation mechanisms for mitomycin C-induced and repressed transcriptome in ADP1 and 17978.

The absolute number of genes induced (A) or repressed (panel B) by MMC in the transcriptome of ADP1 and 17978 is shown. The designation of regulon is represented by the following terms: Neither (genes requiring neither umuDAb nor recA for regulation); Both (genes requiring both umuDAb and recA for regulation); RecA (genes requiring only recA for regulation); UmuDAb (genes requiring only umuDAb for regulation); (A) Many more repressed genes were observed in ADP1 than 17978, with UmuDAb sufficing for this repression in most genes; 17978 repressed genes required either UmuDAb or both UmuDAb and RecA. (B) A greater number of induced genes was observed in 17978 than ADP1, and these genes required either RecA or both RecA and UmuDAb. In comparison, ADP1 induced genes belong to four regulons (Neither, Both, RecA, or UmuDAb).

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60 – 100%) to genes in the Acinetobacter siphovirus Bφ-B1251, which was found in a sewage sample and lysed a carbapenem-resistant A. baumannii clinical isolate [42]. Both CP5 and CP14 were composed of a variety of phages’ genes, with no one species being in the majority. This difference was statistically significant (p<0.05, Fisher’s exact test).

All three prophage regions were within the size range for non-Bacillus siphovirus genomes (14–56 kb [43,44]) and were organized into modules of (in this order): lysogeny/regulation, DNA metabolism, DNA packaging and head, tail, and lysis genes (Figure 1B), which is the same organization as in genomes from the family Siphoviridae [43,45]. This analysis and annotation by the PHAST software, as well as manual characterization and genome size of the three prophage regions, suggested that the 45 kb CP5 was an intact prophage, encoding the requisite morphological (capsid, packaging, tail), DNA replication and lysogeny regulation (including repressors; Table 4) gene products indicative of a functional prophage. However, the 49 kb CP9 and the 22 kb CP14 also contained these genes and may be intact prophages as well. Thus the composition as well as the induction of these prophages differed from the (uninduced) prophage loci present in ADP1, the larger (~53 kb) of which contains only roughly one third of its genes as phage genes (either as conserved hypotheticals or of known function) (Figure 1A), [28], most of which resembled genes from the Family Myoviridae.

We hypothesized that because ~99% of all the genes in these prophages were induced after DNA damage, bacteriophage particles might be produced under these conditions. When 17978 cells were grown in LB medium in the presence of MMC, a decrease in culture turbidity was observed beginning around two hours post-exposure, relative to untreated cells (Figure 5A). Transmission electron microscopy was used to visualize intact phage particles of uniform morphology from filtered supernatants of these cultures in three independent experiments. Morphological analyses of these phages showed them to have a non-enveloped capsid of approximately 57 nm in diameter, and a long, thin (11 nm), flexible tail of approximately 167 nm that possessed tail fibers (Figure 5B). These morphological
features, together with the size, content and organization of the three prophage regions, suggest that the phage particles may belong to the viral family Siphoviridae. Bacteriophages in the Myoviridae family visually resemble siphoviruses but possess a wider and inflexible tail. Furthermore, viruses in the Myoviridae family are lytic, and thus not consistent with the temperate nature of the phages that we observed.

We tested whether these similar-looking bacteriophages represented the products of CP5, CP9, CP14, or a mixture of all three of these prophages, as was suggested by the induction of genes in all three prophage regions. Phage particles were purified away from bacterial fragments and chromosomal DNA in both MMC-treated and untreated cultures by DNase treatment of supernatant that had been 0.22 μm filtered and precipitated. PCR amplification experiments were performed on these DNase-treated, purified samples to determine whether genes from each prophage region were present in the particles we observed. Primers that amplified portions of rumB (from CP5), esvI and umuC A1S_2015 (from CP9), and esvK1 (from CP14) all yielded PCR products only from 3 hour-MMC-treated, purified culture supernatants, but not from untreated, purified culture supernatants, in three independent experiments. This suggests that all three putative prophage regions might produce phage particles when induced by MMC, although it was not unambiguously determined that these particles are encoded by each of the three prophage regions independently, or whether one of these phage, e.g. CP5, might have served as a helper virus for the production of particles containing CP9 or CP14 prophage DNA.

We next tested the hypothesis that the umuD-rumB (A1S_1173-1174) operon that we observed in the phage lysate is responsible for the DNA damage-induced mutagenesis previously observed in this strain [14,32]. Compared to the frequency of rifampin-resistant mutants observed in wild type 17978 cells after UV exposure, a rumB null mutant displayed only approximately ~40% of the rifampin mutation frequency after DNA damage (in four independent experiments). This suggests that if CP5 produced phage particles, these could transduce these genes into a new host and so allow error-prone replication of DNA in this host. However, a similar, partial (~65%) reduction of rifampin resistance frequency in a non-phage encoded umuD (A1S_0636) null mutant was also observed (in six independent experiments). The apparent redundancy of these error-prone polymerases in the DNA damage-inducible mutagenesis occurring in the 17978 strain is likely a reflection of these polymerases being of bacteriophage origin as well as bacterial origin in this species.

Discussion

These transcriptome studies of A. baumannii ATCC 17978 and A. baylyi ADP1 indicated that a genome-wide system of inducing and repressing genes after DNA damage exists in these species. Between 2% and 4% of these species’ genes were induced after mitomycin C treatment, but their distribution throughout the chromosome differed greatly, with localization of most (~90%) of the 17978 genes into three prophages but wide dispersal of ADP1 induced genes throughout the chromosome. There was little overlap in the DNA damage-induced transcriptomes of these organisms with either canonical SOS genes (only 11–17% of which were induced) or each other (only reca, sbb, umuDAb, ddrR, and gst were induced in both species). recA, ssb, and umuD are core SOS genes, whereas gst encodes a member of the glutathione S-transferase (GST) family, which protects against oxidative stress and detoxifies endogenous, xenobiotic and antimicrobial compounds [35]. A. baumannii ATCC 17987, like many proteobacteria, possesses multiple (9) gst genes [46], as does A. baylyi ADP1 [28]. The induced gst genes (A1S_0408 and ACIAD0445) share 69% amino acid identity, and are present in a highly syntenic chromosomal location in these two species, allowing for the possibility that A1S_0408 and ACIAD0445 may be members of the GST family that participate in the DNA damage response.

Besides a subset of the canonical SOS genes, stress proteins and chaperones, ADP1 induced the genes of a CRISPR/Cas system, which are bacterial adaptive immunity/defense modules. The cell processes foreign, e.g. bacteriophage, DNA molecules and forms a
Table 4. Description of gene functions in order of appearance in each prophage in 17978.

| Phage Region | A1S | Name/Function | Category |
|--------------|-----|---------------|----------|
| CP5          | 1140 | ribonuclease   | DNA Metabolism/Replication |
|              | 1141 | global regulatory protein | Lysogeny/Regulation |
|              | 1142 | aspartate kinase | Moron |
|              | 3603 | hypothetical protein | Phage hypothetical protein |
|              | 3604 | hypothetical protein | Phage hypothetical protein |
|              | 3605 | hypothetical protein | Phage hypothetical protein |
|              | 3606 | hypothetical protein | Phage hypothetical protein |
|              | 1143 | hypothetical protein | Hypothetical protein |
|              | 1144 | repressor | Lysogeny/Regulation |
|              | 1145 | putative Cro protein | Lysogeny/Regulation |
|              | 3607 | Rha family transcriptional regulator | Lysogeny/Regulation |
|              | 3608 | hypothetical protein | Phage hypothetical protein |
|              | 1146 | methytransferase | DNA Metabolism/Replication |
|              | 1147 | site-specific DNA methylase-like protein | DNA Metabolism/Replication |
|              | 3609 | hypothetical protein | Phage hypothetical protein |
|              | 3610 | hypothetical protein | Hypothetical protein |
|              | 3611 | Hypothetical protein | Phage hypothetical protein |
|              | 3612 | HNH endonuclease | DNA Metabolism/Replication |
|              | 3613 | hypothetical protein | Phage hypothetical protein |
|              | 1148 | hypothetical protein | Phage hypothetical protein |
|              | 3614 | hypothetical protein | Phage hypothetical protein |
|              | 1149 | hypothetical protein | Phage hypothetical protein |
|              | 1150 | hypothetical protein | Phage hypothetical protein |
|              | 1151 | phage protein | Phage hypothetical protein |
|              | 1152 | terminase, large subunit | Capsid/DNA packaging |
|              | 1153 | portal protein | Capsid/DNA packaging |
|              | 1154 | phage head morphogenesis protein | Capsid/DNA packaging |
|              | 3615 | hypothetical protein | Hypothetical protein |
|              | 3616 | hypothetical protein | Hypothetical protein |
|              | 1155 | putative head protein | Capsid/DNA packaging |
|              | 1156 | hypothetical protein | Phage hypothetical protein |
|              | 1157 | major capsid protein | Capsid/DNA packaging |
|              | 1158 | putative signal peptide | Moron |
|              | 1159 | hypothetical protein | Phage hypothetical protein |
|              | 3617 | hypothetical protein | Hypothetical protein |
|              | 1160 | hypothetical protein | Hypothetical protein |
|              | 1161 | hypothetical protein | Hypothetical protein |
|              | 1162 | hypothetical protein | Hypothetical protein |
|              | 3618 | hypothetical protein | Hypothetical protein |
|              | 1163 | hypothetical protein | Hypothetical protein |
|              | 3619 | hypothetical protein | Hypothetical protein |
|              | 1164 | tail tape measure protein | Tail Morphogenesis |
|              | 1165 | tail tape measure protein | Tail Morphogenesis |
|              | 1166 | hypothetical protein | Phage hypothetical protein |
|              | 1167 | tail assembly structural protein | Tail Morphogenesis |
|              | 1168 | hypothetical protein | Phage hypothetical protein |
|              | 3620 | hypothetical protein | Phage hypothetical protein |
|              | 1169 | putative tail protein | Tail Morphogenesis |
|              | 1170 | putative tail protein | Tail Morphogenesis |
|              | 3621 | hypothetical protein | Phage hypothetical protein |
Table 4. Cont.

| Phage Region | A1S       | Name/Function                                      | Category                      |
|--------------|-----------|---------------------------------------------------|-------------------------------|
|              | 1171      | hypothetical protein                              | Phage hypothetical protein    |
|              | 1172      | IS903 transposase                                 | Transposase/Insertion sequence|
|              | 1173      | rumB, error-prone legion bypass DNA polymerase V  | Error-prone polymerase/Moron  |
|              | 1174      | umuD                                             | Error-prone polymerase/Moron  |
|              | 3622      | hypothetical protein                              | Phage hypothetical protein    |
|              | 1175      | phage integrase                                   | Lysogeny/Regulation           |
| CP9          | 2040      | putative integrase                                | Lysogeny/Regulation           |
|              | 3779      | hypothetical protein                              | Hypothetical protein          |
|              | 3778      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3777      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3776      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2039      | putative phage nucleotide-binding protein         | DNA Metabolism/Replication     |
|              | 2038      | hypothetical protein                              | Hypothetical protein          |
|              | 3775      | hypothetical protein                              | Hypothetical protein          |
|              | 3774      | hypothetical protein                              | Hypothetical protein          |
|              | 2037      | esvI/putative repressor cl                        | Lysogeny/Regulation           |
|              | 3773      | hypothetical protein                              | Hypothetical protein          |
|              | 3772      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2036      | DNA cytosine methyltransferase                    | DNA Metabolism/Replication     |
|              | 3771      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3770      | hypothetical protein                              | Hypothetical protein          |
|              | 2035      | HNH nuclease                                      | DNA Metabolism/Replication     |
|              | 3769      | hypothetical protein                              | Hypothetical protein          |
|              | 3768      | hypothetical protein                              | Hypothetical protein          |
|              | 2034      | putative antirepressor                            | Lysogeny/Regulation           |
|              | 2033      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2032      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2031      | phage protein                                     | Phage hypothetical protein    |
|              | 2030      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2029      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2028      | phage head morphogenesis protein                  | Capsid/DNA Packaging          |
|              | 3767      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3766      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2027      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2026      | major capsid protein                              | Capsid/DNA Packaging          |
|              | 2025      | major capsid protein                              | Capsid/DNA Packaging          |
|              | 3765      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3764      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2024      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3763      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2023      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3762      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3761      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2022      | putative tail fiber                               | Tail Morphogenesis            |
|              | 2021      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3760      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2020      | hypothetical protein                              | Phage hypothetical protein    |
|              | 2019      | hypothetical protein                              | Phage hypothetical protein    |
|              | 3759      | putative lipoprotein                              | Phage hypothetical protein    |
| Phage Region | A1S or CP14 | Name/Function | Category |
|-------------|-------------|---------------|----------|
|             | 2018        | tail tape measure protein | Tail Morphogenesis |
|             | 3758        | hypothetical protein | Phage hypothetical protein |
|             | 3756        | hypothetical protein | Hypothetical protein |
|             | 3757        | hypothetical protein | Phage hypothetical protein |
|             | 2017        | tail fiber | Tail Morphogenesis |
|             | 3755        | holin | Lysis |
|             | 2016        | lysozyme | Lysis |
|             | 3754        | hypothetical protein | Phage hypothetical protein |
|             | 2015        | umuC, error-prone DNA polymerase | Error-prone polymerase |
|             | 2014        | hypothetical protein | Phage hypothetical protein |
|             | 3755        | holin | Lysis |
|             | 3756        | hypothetical protein | Hypothetical protein |
|             | 3757        | hypothetical protein | Phage hypothetical protein |
|             | 2017        | tail fiber | Tail Morphogenesis |
|             | 3755        | holin | Lysis |
|             | 2016        | lysozyme | Lysis |
|             | 3754        | hypothetical protein | Phage hypothetical protein |
|             | 2015        | umuC, error-prone DNA polymerase | Error-prone polymerase |
|             | 2014        | hypothetical protein | Phage hypothetical protein |
| CP14 | 1580 | integrase | Lysogeny/Regulation |
|       | 3683        | hypothetical protein | Hypothetical protein |
|       | 3684        | hypothetical protein | Hypothetical protein |
|       | 1581        | putative methyltransferase | DNA Metabolism/Replication |
|       | 3685        | hypothetical protein | Hypothetical protein |
|       | 3686        | repressor | Lysogeny/Regulation |
|       | 3687        | hypothetical protein | Hypothetical protein |
|       | 1582        | transcriptional regulator Cro/Cl family | Lysogeny/Regulation |
|       | 1583        | hypothetical protein | Hypothetical protein |
|       | 1584        | hypothetical protein | Hypothetical protein |
|       | 1585        | putative replicative DNA helicase | DNA Metabolism/Replication |
|       | 3688        | hypothetical protein | Hypothetical protein |
|       | 3689        | hypothetical protein | Hypothetical protein |
|       | 3690        | hypothetical protein | Hypothetical protein |
|       | 3691        | hypothetical protein | Hypothetical protein |
|       | 3692        | hypothetical protein | Hypothetical protein |
|       | 3693        | hypothetical protein | Phage hypothetical protein |
|       | 3694        | hypothetical protein | Phage hypothetical protein |
|       | 3695        | hypothetical protein | Hypothetical protein |
|       | 3696        | hypothetical protein | Hypothetical protein |
|       | 1586        | esvK1/ethanol-stimulated virulence protein | DNA Metabolism/Replication |
|       | 3697        | HNH nuclease | DNA Metabolism/Replication |
|       | 3698        | hypothetical protein | Hypothetical protein |
|       | 1587        | esvK2/terminase | Capsid/DNA packaging |
|       | 1588        | large terminase | Capsid/DNA packaging |
|       | 1589        | portal protein | Capsid/DNA packaging |
|       | 1590        | Pro-head protease | Capsid/DNA packaging |
|       | 1591        | putative head major capsid protein | Capsid/DNA packaging |
|       | 3699        | hypothetical protein | Hypothetical protein |
|       | 1592        | head/tail adapter protein | Capsid/DNA packaging |
|       | 1593        | hypothetical protein | Phage hypothetical protein |
|       | 1594        | hypothetical protein | Phage hypothetical protein |
|       | 1595        | major tail subunit | Tail Morphogenesis |
|       | 3700        | hypothetical protein | Hypothetical protein |
|       | 3701        | putative lipoprotein | Phage hypothetical protein |
|       | 1596        | tail length tape measure protein | Tail Morphogenesis |
|       | 1597        | tail tape measure protein | Tail Morphogenesis |
|       | 3702        | hypothetical protein | Phage hypothetical protein |
|       | 1598        | putative tail fiber protein | Tail Morphogenesis |
### Table 4. Cont.

| Phage Region | A1S | Name/Function          | Category   |
|--------------|-----|------------------------|------------|
| 1599         |     | hypothetical protein   | Phage      |
| 3703         |     | hypothetical protein   | Phage      |
| 3704         |     | hypothetical protein   | Lysis      |
| 1600         |     | lysozyme               | Lysis      |

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**Figure 5. Mitomycin C treatment induces production of bacteriophage particles in 17978.** (A) Overnight LB cultures of 17978 cells were diluted into fresh LB medium and grown for 0.75 hours before addition of 2 μg/mL MMC. After approximately two hours of MMC treatment, the optical density leveled off and decreased slightly but continued to increase in the absence of MMC treatment. Error bars represent standard error of the mean from three independent experiments. (B) Electron micrograph of bacteriophage particles at 100,000× magnification, showing polyhedral capsid, long, flexible tail and tail fibers. Results shown are representative of three independent experiments producing and imaging bacteriophage particles.

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CRISPR array locus in the chromosome composed of short segments of these DNA sequences [47]. The next time similar DNA molecules enter the cell, Cascade proteins (cas gene-encoded, typically adjacent to the CRISPR repeat locus) and transcribed CRISPR sequences bind to and cleave the incoming foreign DNA. The *A. baylyi* ADP1 Type I-F CRISPR/Cas locus consists of the Cascade proteins encoded by *cas3/cas2* (ACIAD2477), as well as two conserved hypothetical genes, *cas5*, *cas3*, *cas6*, and *cas1* (ACIAD2479-2484, which were induced by MMC). It is intriguing that in *A. baylyi* ADP1 cells, which are naturally competent for the uptake of and transformation with DNA [15], this CRISPR/Cas defense against foreign DNA appears to be functional. Short transcribed CRISPR RNA molecules (crRNA), identical to those comprising the CRISPR repeats adjacent to the induced ACIAD2479-2484 genes, accumulate in ADP1 cells after treatment with nalidixic acid [48], a well-known inducer of the SOS response. The dependence of these crRNA molecules’ formation on new protein synthesis [48] is consistent with induction of the *cas* genes that we observed. A link between a CRISPR/Cas system and DNA repair has been observed in *E. coli*, where the Casi nuclease YgbT acts on both branched DNAs and in antiviral immunity [49]. However, to our knowledge, this is the first evidence of transcriptional induction of a CRISPR/Cas system gene by DNA damage. To the limited extent that CRISPR/Cas genes’ expression has been studied, a constitutive level has been assumed [48], but the uninduced level of A1S_2479-2484 expression is modest, being below the average uninduced level of the 66 induced genes, but approximately four times the detection threshold of the RNASeq experiments.

Our data are largely consistent with those observed in recent microarray studies of *A. baumannii* ATCC 17978 in which 39 genes were induced more than 1.5-fold after MMC treatment [19], with 77% of that study’s genes also induced in our experiments. The greater number of induced genes observed in our study (152), as well as the variation in the specific identity of the induced genes may be because of the different methodologies used (RNA-Seq vs microarray), and also because Aranda *et al.* used a rich medium source (LB broth), a shorter induction time of two hours, and one-quarter the amount of MMC as in this study. The invariant conservation of the induction of all error-prone polymerases and polymerase components in this and other studies [19,32], however, supports the centrality of these genes to the DNA damage response of this species.

Further transcriptional profiling of *umuDb* and *recA* mutant strains of both species after MMC treatment allowed determination of the roles of these putative regulators in the DNA damage responses. In the DNA damage-induced transcriptomes, *recA* was required for the induction of only 38% of the ADP1 induced genes, but virtually all of the 17978 induced genes, which is consistent with both the known SOS response mechanism [4] and the involvement of *recA* in antimicrobial resistance, general stress responses, and virulence in 17978 [23]. This *recA* dependence is also consistent with the repression of the prophage genes by a prophage-encoded repressor [41] as opposed to a LexA-like, UmuDAb-mediated repression of these genes. However, we observed 9-19% of each of the three prophage genomes required *umuDb* for gene induction in addition to *recA*, which argues against a solitary action of RecA-facilitated autocleavage of a prophage repressor in the response we observed. The action of UmuDAb, a potential LexA homolog, was complex in both species, playing a role in only 44% of ADP1 induced genes, and in 16% of 17978 induced genes, including both those encoded in prophages and in the chromosome. The large number of repressed genes in the DNA damage transcriptomes, especially of ADP1 (Figure 2B), was unexpected, with the repression action of UmuDAb being consistent with its involvement in the repression of the vast majority of these genes in ADP1, although its action may be indirect rather than direct.

The de-repression that we observed of *ddrR* and all *umuDC* alleles in a null *umuD* mutant is consistent with recent observations that UmuDAb binds to, and regulates, the promoters of these genes in *A. baumannii* ATCC 17978 [19], although those studies used a *umuDb* insertion mutant and not a null mutant. However, our genome-wide profiling of *umuDb* regulation of induced genes found that unlike for the *umuDC* alleles, the induction of the majority (83.5%) of all genes in 17978 was *umuDb*-independent. Either UmuDAb is not the sole LexA-like repressor in this species, or has a mechanism of action unlike LexA, because a LexA-regulated regulon of DNA damage-induced genes would have become de-repressed in the absence of DNA damage, which was not observed (except for the *umuDC* and *ddrR* genes). Furthermore, *umuDb* was required for the induction of genes that are not error-prone polymerases and which were encoded in prophage regions (Table 3). These data suggest that UmuDAb does not serve as a direct replacement of LexA for the entire DNA damage regulon in this genus, instead serving a more specialized role in repressing error-prone polymerases. This specialized UmuDAb role invokes an additional DNA damage-related repressor to regulate gene expression after DNA damage, which is consistent with the failure of RecA to regulate its own induction, seen both in this study and previously for *A. baylyi* ADP1 [16] and *A. baumannii* [32].

In having multiple *umuDb*-dependent and -independent regulons, the behavior of *Acinetobacter* in regulating their genes after DNA damage is more like its closer pseudomonad relatives, which contain multiple regulons of DNA damage-induced genes involving different (LexA) repressor proteins [12], than it is to enteric bacteria such as *E. coli*. These *Acinetobacter* species, like *P. aeruginosa*, also repressed many more genes than they induced in response to DNA damage, and both genera repressed multiple canonical SOS genes in a *lexA*-independent manner (*recG* in ADP1, and *holB* and *recC* in both ADP1 and 17978), and induced *mdrAB* and prophage genes [34].

Our observation of the 17978 strain possessing DNA damage-inducible bacteriophages that encode mutation-inducing (error prone) polymerase genes may hold significant implications for the evolution of virulence and antibiotic resistance in related strains. CP5 encodes the *umuDrumB* operon, which this study found to be responsible for at least half of the DNA damage-induced mutagenesis, while CP9 encodes *AIS_2015*, annotated as an “error-prone lesion bypass DNA polymerase V” that might also contribute to mutagenesis after DNA damage [32]. Multiple DNA damage-inducing agents–UV-C exposure [14,32] as well as methyl methanesulfonate, desiccation, and ciprofloxacin [32]–are capable of inducing mutagenesis (as measured by rifampin resistance) in *A. baumannii* ATCC 17978 and AB0057 [14]. *A. baumannii* strains AB0057 and 3909 also contain CP5 that encodes the *umuDrumB* genes [38], while *A. baumannii* ATCC 19606 and D2797797, strains not investigated by DiNocera *et al.*, also possess a very similar CP5-like prophage region that encodes *umuDrumB* (Figure S1). This indicates the possibility of a widespread mechanism in this species for spread of these error-prone polymerase genes in response to multiple stimuli. Virulence-associated genes such as *esvK1* and *esvK2* (encoded in CP14), and *esvL* (encoded in CP9) that contributed to ethanol-stimulated virulence in a model of *C. elegans* infection by the 17978 strain [27] are also encoded by these prophages and could contribute to the evolution of strains through transduction by bacteriophages that may be produced from, or
encapsidate, the genomes of CP5, CP9, or CP14, although these phages have not yet been shown to infect other hosts.

The overall patterns of UmuDAb and RecA usage in these species suggests that diverse mechanisms exist in A. baumannii ADPI for the repression and induction of genes, which include a regulon induced by either UmuDAb or RecA. In contrast, A. baumannii ATCC 17978 almost universally depends on RecA (as well as UmuDAb) but also uses additional, unknown repressors and/or regulators, possibly of phage origin, in addition to UmuDAbs. These species therefore offer robust model systems in which to study the processes of gene regulation after DNA damage, with A. baumannii additionally posing a relevant biological problem in its possible dissemination of error-prone polymerases.

Supporting Information

Figure S1 CP5-like prophage regions present in A. baumannii strains. The three to-scale diagrams indicate CP-like prophage regions present in A. baumannii strains ATCC 19606 and D1279779. Analysis and image production was performed using the PHAST webserver, with the color-coding indicating the likely function assigned to each coding sequence. The numbered bar indicates the nucleotide number in the genome, with coding regions in the three forward frames shown above the bar and coding regions in the three reverse frames shown below the bar for each strain.

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Table S1 PCR primers used in constructing umuDAb, umuD, and rumB mutants of A. baumannii ATCC 17978.

Table S2 Primers used in RT-qPCR experiments in A. baumannii ADPI.

Table S3 Primers used in RT-qPCR experiments in A. baumannii ATCC 17978.

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Author Contributions

Conceived and designed the experiments: JMH JCF. Performed the experiments: ANG JMH JCF. Analyzed the data: JMH TAW ANG. Wrote the paper: JMH ANG.
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