A plasmid-encoded UmuD homologue regulates expression of *Pseudomonas aeruginosa* SOS genes

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The *Pseudomonas aeruginosa* plasmid pUM505 contains the *umuDC* operon that encodes proteins similar to error-prone repair DNA polymerase V. The *umuC* gene appears to be truncated and its product is probably not functional. The *umuD* gene, renamed *umuDpR*, possesses an SOS box overlapped with a Sigma factor 70 type promoter; accordingly, transcriptional fusions revealed that the *umuDpR* gene promoter is activated by mitomycin C. The predicted sequence of the UmuDpR protein displays 23% identity with the *Ps. aeruginosa* SOS-response LexA repressor. The *umuDpR* gene caused increased MMC sensitivity when transferred to the *Ps. aeruginosa* PAO1 strain. As expected, PAO1-derived knockout *lexA* mutant PW6037 showed resistance to MMC; however, when the *umuDpR* gene was transferred to PW6037, MMC resistance level was reduced. These data suggested that UmuDpR represses the expression of SOS genes, as LexA does. To test whether UmuDpR exerts regulatory functions, expression of PAO1 SOS genes was evaluated by reverse transcription quantitative PCR assays in the *lexA*– mutant with or without the pUC_umuD recombinant plasmid. Expression of *lexA*, *imuA* and *recA* genes increased 3.4–5.3 times in the *lexA*– mutant, relative to transcription of the corresponding genes in the *lexA*+ strain, but decreased significantly in the *lexA*–/*umuDpR* transformant. These results confirmed that the UmuDpR protein is a repressor of *Ps. aeruginosa* SOS genes controlled by LexA. Electrophoretic mobility shift assays, however, did not show binding of UmuDpR to 5’ regions of SOS genes, suggesting an indirect mechanism of regulation.

**INTRODUCTION**

pUM505 is a conjugative plasmid, originally isolated from a clinical *Pseudomonas aeruginosa* strain (Cervantes et al., 1990), which confers resistance to chromate and inorganic mercury (reviewed by Ramírez-Díaz et al., 2008). Sequence analysis revealed that pUM505 carries several putative adaptive genes, including *umuDC* genes, whose products are similar to the subunits of error-prone repair DNA polymerase V (Pol V), the *pdi* gene, associated with the protection of proteins from oxidative damage, and the *virB4*, *virD4* and *hop* genes, which are associated with virulence (Ramírez-Díaz et al., 2011).

Expression of the chromosomal *Escherichia coli* *umuD* gene is upregulated as part of the SOS response to DNA damage (Nohmi, 2006). UmuD is initially produced as a 139 aa protein, which subsequently cleaves off its N-terminal 24 aa residues in a reaction dependent on a RecA/single-stranded DNA complex, giving rise to UmuD’ (Nohmi, 2006). The two forms of the *umuD* gene product play different roles in the cell. UmuD is implicated in a primi-tive DNA-damage checkpoint (*UmuD*2C complex) and prevents DNA polymerase IV-dependent −1 frameshift mutagenesis (Kivisaar, 2010), while the cleaved UmuD’ form facilitates UmuC-dependent mutagenesis via formation of DNA polymerase V after its association with the UmuC protein (the UmuD’2C complex) (Patel et al., 2010).

**Abbreviations**: EMSA, electrophoretic mobility shift assay; MMC, mitomycin C; Pol V, DNA polymerase V; qPCR, quantitative PCR; RT-PCR, reverse transcription PCR

Two supplementary tables and two supplementary figures are available with the online Supplementary Material.
However, the soil bacterium *Acinetobacter baylyi* strain ADP1 contains a chromosomal *umuDaB-umuC* operon that is unusual in content, regulation and function as compared with the *E. coli* homologue (Hare et al., 2006). The *umuDaB* gene encodes an extra 59 aa N-terminus region that is not encoded by other *umuD* genes, and the *umuC* gene is incomplete (Hare et al., 2006; Fig. 1a). This operon does not contain an SOS box, and its expression is constitutive (Hare et al., 2006). *UmuDaB* carries out self-cleavage in a RecA-dependent manner after cells experience diverse forms of DNA damage, and shares features with both the Pol V component UmuD and the

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**Fig. 1.** (a) Comparison of *umuDC* operons. Coding regions of *umuDC* genes from *E. coli* K-12, plasmid pUM505 and *A. baylyi* ADP1 are indicated by large open arrows, with the size of predicted polypeptides (aa) indicated below; *umuC* corresponds to a truncated *umuC* gene. The location of putative promoter sequences is marked by bent vertical arrows and SOS boxes are indicated by small rectangles. Percentages of amino acid sequence identity/similarity of the compared proteins are indicated in parentheses; shaded areas were not considered for the alignments. Black arrows signal the locations of primers, as described in the text. Information on the *umuDC* genes of *E. coli* and *A. baylyi* was drawn from Kitagawa et al. (1985) and Hare et al. (2006), respectively. (b) Multiple sequence alignment of UmuD homologues. Alignments include sequences of UmuDpR from the pUM505 plasmid. LexA Paer, *Ps. aeruginosa* PA01; LexA Ecol, *E. coli* K-12; UmuDaB Abau, *A. baumannii* ATCC 17978; UmuDaB Abay, *A. baylyi* ADP1; UmuD Ecol, *E. coli* K-12; RumA Pret, *Providencia rettgeri* R391 plasmid; MucA Smar, *Serratia marcescens* R471a plasmid; MucA Styp, *Salmonella typhimurium* R934 plasmid. Shaded black and grey areas correspond to identical and similar residues, respectively. The (Ala/Cys)-Gly cleavage site residues and Ser-Lys active site residues are indicated by filled and open circles, respectively (Luo et al., 2001). The Leu-Arg motif that is required for UmuD self-cleavage in *E. coli* is indicated by a rectangle (Sutton et al., 2001).
LexA repressor (Hare et al., 2012). Recent work demonstrated that in the opportunistic pathogen Acinetobacter baumannii strain ATCC 17978, UmuDAb binds to and represses the promoters of umuDC homologues; thus, it might serve as a LexA analogue for this genus (Aranda et al., 2013). The significance of the regulatory role of UmuDAb is emphasized by the absence of a LexA homologue in Acinetobacter (Aranda et al., 2013).

Because the majority of Ps. aeruginosa strains lack umuDC chromosomal genes, the umuDC operon from plasmid pUM505 may function as a regulator of the expression of genes associated with the DNA damage response in this bacterium. The objective of this work was to characterize the function of pUM505 umuDC genes.

We report here that the product of the pUM505 umuD gene is able to repress expression of SOS genes, and suggest that possession of this anti-SOS factor can be evolutionarily advantageous for the pUM505 plasmid.

**METHODS**

**Bacterial strains and culture conditions.** Ps. aeruginosa PAO1 (from Dr B. Iglewski’s collection; Li et al., 2007) and E. coli W3110 (Hayashi et al., 2006) are prototroph, standard strains that were used as hosts for recombinant plasmids. The PAO1-derived mutant strain PW6037 (lexA::IspHoa/Abp) (obtained from the Ps. aeruginosa Mutant Library; Jacobs et al., 2003) was used for analysis of the expression of SOS genes. E. coli BL21-Codon-Plus(DE3)-RP (StrataGene) was used for protein overexpression.

Culture media employed were nutrient broth (NB) or Luria–Bertani broth (LB; 1.5 % agar for solid medium) (Green & Sambrook, 2012). The cloning process of the constructs was verified by genetic techniques and sequence analysis.

**Genetic techniques and sequence analysis.** General molecular genetic techniques were used according to standard protocols (Green & Sambrook, 2012). The cloning process of the constructs was verified by DNA sequencing carried out at the Department of Genetics, Cinvestav, Irapuato, Mexico. Protein sequence alignments were calculated with the CLUSTAL W algorithm and BioEdit Sequence Alignment Editor Software. Sequence similarities in protein and DNA databases were searched for using BLASTP and BLASTX programs (http://blast.ncbi.nlm.nih.gov/blast.cgi). Potential promoter sequences were searched for using the Neural Network Promoter Prediction (http://www.fruitfly.org/seq_tools/promoter.html). Transcription-factor binding sites were determined using Virtual Footprint and PRODORIC (http://www.prodoric.de/vfp/vfp_promoter.php).

**Reverse transcription (RT)-PCR assays.** Cells were grown at an OD600 of 0.6 (mid-exponential phase) in NB at 37 °C with shaking. Total RNA was isolated by using Tri reagent (Molecular Research Center). DNA was removed with RQ1 RNase-free DNase (Promega), and RNA was quantified by spectrophotometric analysis at 260 nm. RT-PCR was performed with 50 ng of RNA samples and the one-step Access RT-PCR System kit (Promega). The oligonucleotides used, umuD_F and umuD_R (Table S1, available in the online Supplementary Material), amplify a 222 bp fragment containing the overlap region of the umuDpR and umuC genes (Fig. 1a). Positive and negative controls were performed with PCR master mix (Promega) and the pairs of primers described in Table S1 using plasmid DNA or total RNA as templates, respectively.

**Transcriptional fusions.** A 378 bp DNA fragment containing the putative umuDpR gene 5′ regulatory region was amplified by PCR from purified pUM505 plasmid DNA employing the oligonucleotides listed in Table S1. Primers were located 243 bp upstream and 135 bp downstream of the umuDpR start codon, respectively. PCR fragments were purified and cloned into the pET12/Blunt vector (Merck). Recombinant plasmids were transferred by electroporation into E. coli strain W3110, and transformants were selected on LB agar plates with ampicillin. DNA fragments from recombinant plasmids were obtained by digestion with EcoRI and BamHI endonucleases and subcloned into the corresponding sites of the pLP170 vector, which possesses a promoterless lacZ reporter gene (Preston et al., 1997). Recombinant plasmid pLP_umuDC was then transferred by electroporation into the Ps. aeruginosa strain PAO1 and transformants were selected on LB agar plates with carbenicillin, β-Galactosidase activities were determined by utilizing the chromogenic substrate ONPG (Sigma) in permeabilized cells, as described previously (Acosta-Navarrete et al., 2014). Enzyme activities were corrected by subtracting the values obtained from cultures of cells bearing the pLP170 vector without insert.

**Cloning of the umuDpR-umuC genes.** The umuDpR-umuC genes, or the individual umuDpR gene, were obtained by PCR from pUM505 plasmid DNA using specific primers (Table S1). PCR fragments were purified, cloned into the pET12/Blunt vector, and transferred into the E. coli strain W3110, as described in the previous section. DNA fragments were obtained by digestion with BamHI and HindIII, and subcloned into the corresponding sites of the pUCP20 binary vector (West et al., 1994). Recombinant plasmids pUC_umuDC or pUC_umuD were transferred into the Ps. aeruginosa strain PAO1, as described in the previous section.

The umuDpR gene coding region was cloned into the pET12/Blunt vector as described in the previous paragraph. DNA fragments were obtained by digestion with BamHI and EcoRI and subcloned into the corresponding sites of the pTrcHis2A expression vector (Invitrogen), which provides a 6-His tag at the C-terminal end of the cloned product. Recombinant plasmid pTrcUmuD-His, encoding a tagged UmuDpR-His protein of 174 aa residues (19.2 kDa), was transferred into E. coli BL21-Codon-PlusBL21(DE3)-RP, selecting transformants on LB agar plates with ampicillin.

**Susceptibility tests.** Bacteria were grown by diluting 1:100 overnight cultures in tubes with 4 ml NB with increasing amounts of mitomycin C (MMC) (Sigma). Cultures were incubated for 18 h at 37 °C with shaking and growth was measured at OD600.

cDNA synthesis and quantitative PCR (qPCR). For expression measurements, total RNA was isolated and quantified as described in the section RT-PCR. cDNA synthesis was performed with M-MLV reverse transcriptase (Promega) and random hexamers according to the manufacturer’s instructions. The expression analysis of SOS genes was performed by qPCR using the comparative Ct method (ΔΔCt) with a StepOne Plus Real-Time PCR System (Applied Biosystems). The reactions were carried out with a USB VeriQuest SYBR Green qPCR Master Mix according to the manufacturer’s instructions. qPCR primers (Table S1) were designed using Biosearch Technologies software (https://www.biosearchtech.com/support/applications/realtime.designtool-software). Relative expression of SOS genes was normalized with expression values obtained from the rpoB gene, which encodes the RNA polymerase β subunit (Qi et al., 2001). Specificity of PCR assays was determined from dissociation curves generated after the qPCRs. Appropriate positive and negative controls were included in every test run. The qPCR data were obtained from three
independent experiments performed in duplicate. The results are reported as the means ± se.

**Electrophoretic mobility shift assays (EMSA).** Overnight cultures of *E. coli* BL21-Codon-Plus(DE3)-RP (pTrcUmuD-His) were diluted 1:100 in 100 ml of LB containing 0.1 mM IPTG and incubated for 18 h at 37 °C with shaking. Protein purification was performed essentially as described by Watanabe & Takada (2004). Cells were disrupted by sonic oscillation to clarity and debris was removed by centrifugation. The supernatant was loaded onto a Nickel–NTA resin packed into a column; UmuDpR-His (19 kDa) was recovered by elution with 250 mM imidazole and dialysed as described by Aranda et al. (2008). Purification was monitored by 14 % SDS-PAGE.

For EMSA, DNA fragments of about 400 bp containing the 5’ regions of each of the SOS genes were amplified by PCR using specific primers (Table S1). Purified DNA fragments (100 ng) and varied amounts of purified UmuDpR-His protein were mixed in binding buffer [10 mM Tris/HCl (pH 8.0), 10 mM HEPES, 50 mM KCl, 1 mM EDTA, 5 % glycerol, 0.5 mM DTT and 0.1 mg BSA ml⁻¹] and incubated for 30 min at room temperature. Binding reaction mixtures were loaded onto 6 % native PAGE gels, separated at 100 V for 80 min in TAE buffer (Green & Sambrook, 2012) and stained with 0.01 % ethidium bromide for 30 sec. The Ps. aeruginosa PA01 LiuR transcriptional regulator (ORF PA1016) and a DNA fragment containing the liuR gene 5’ regulatory region, a gift from Dr J. Campos-Garcia, were used as positive control of the gel retardation assays.

**RESULTS AND DISCUSSION**

**umuDC genes of the pUM505 plasmid.**

Owing to annotation errors in the nucleotide sequence of the pUM505 *umuD* (named as *umuDpr*, see later), and in the *umuC* reading frames previously reported (Ramírez-Diaz et al., 2011), we identified new start codons for each gene, which are now predicted to encode proteins of 143 aa and 46 aa, respectively (Fig. 1a).

UmuDpR of pUM505 exhibited sequence identities of 43 % to *E. coli* K-12 UmuD, the best studied UmuD protein, a component of DNA polymerase Pol V (Kitagawa et al., 1985) (Fig. 1a), and of 41 % to *A. baylyi* ADP1 UmuDAb; the *A. baylyi* UmuDAb protein is unusual because it possesses an extra 59 aa N-terminal region (Hare et al., 2006) (Fig. 1a) and has been shown to function as a transcriptional regulator (Hare et al., 2012).

UmuDpR is more similar to the *E. coli* UmuD protein and its homologues RumA and MucA (35–43 % amino acid identity) than to the LexA repressor (23 and 22 % identity to the *Ps. aeruginosa* PA01 and *E. coli* K-12 homologues, respectively) (Fig. 1a). Moreover, no similarity exists between the DNA-binding N-terminal domain of LexA and the same region of UmuDpR (Fig. 1b). However, UmuDpR possesses conserved residues (Fig. 1b) that are required for RecA-facilitated self-cleavage in UmuD (the Pol V subunit) (Sutton et al., 2001), the LexA repressor (Luo et al., 2001) and the UmuDab regulator (Hare et al., 2006, 2012). The mechanism by which these proteins are cleaved is similar in that the active site contains a Ser-Lys dyad and the cleavage site is the dipeptide sequence (Ala/Cys)-Gly (Hare et al., 2006) (Fig. 1b). However, a two-amino-acid motif (Leu-Arg) required for efficient UmuD self-cleavage in *E. coli* (Sutton et al., 2001) is not present in UmuDpR (Fig. 1b); this region is more similar to the corresponding sequences of the LexA and UmuDAb transcriptional regulators. These data suggested that UmuDpR may also function as a transcriptional regulator; for this reason, the pUM505 *umuD* gene was herein renamed *umuDpr* (for *umuD* plasmid Regulator).

The pUM505 *umuC* gene encodes a putative protein that is much smaller than its homologues with established function, such as *E. coli* UmuC (Kitagawa et al., 1985) (Fig. 1a). pUM505 UmuC presents sequence identities of 38 % to the N-terminal 63 aa of *E. coli* UmuC and of 44 % to *A. baylyi* UmuC*, another small polypeptide that is considered a truncated protein (Hare et al., 2006) (Fig. 1a). Thus, the *umuC* gene of pUM505 also appears to encode a truncated product that will be referred to as UmuC*, which is probably not functional (see later).

**umuDpr-umuC* genes constitute an operon.

In the majority of bacteria, *umuDC* genes, as well as their homologues, are usually adjacent, their coding regions overlap, and they form operons (Ippoliti et al., 2012). The reading frames of *umuDpr-umuC* genes of pUM505 overlap by 14 nt, suggesting that they form an operon (Fig. 1a). Furthermore, *in silico* analysis of the 5’ regions of the *umuDpr* and *umuC* genes identified a potential promoter only for *umuDpr* (Fig. 1a). To confirm the transcriptional linkage of *umuDpr* and *umuC* genes, RT-PCR analysis was performed. Primers were designed to amplify cDNA synthesized from a transcript spanning the reading frame overlap of *umuDpr-umuC* genes (Fig. 1a). When total RNA from *Ps. aeruginosa* PA01 (pUM505) was analysed by RT-PCR, a faint but consistent 200 bp band was detected (Fig. 2a, lane 2). The corresponding DNA fragment was also detected in positive, but not negative, controls (Fig. 2a, lanes 3 and 4, respectively). These data demonstrate that the *umuDpr* and *umuC* genes from the pUM505 plasmid are cotranscribed into a bicistronic mRNA, and suggest that they form an operon whose expression depends on the *umuDpr* putative promoter.

**Induction of the *umuDpr* gene by MMC**

The putative regulatory region of the *umuDpr* gene presents a potential promoter with consensus −35 and −10 boxes related to general Sigma factor 70 type promoter sequences (Fig. 2b). Sequence analysis of the 5’ region of the *umuDpr* gene identified a putative transcription-factor binding region with similarity to an SOS box, which partially overlaps with the −10 box of the promoter sequence (Fig. 2b). The SOS box of *umuDpr* exhibits a perfect match with regulatory regions of the *umuDC* genes of *E. coli* (Kitagawa et al., 1985) and of *rulAB* homologues from *Pseudomonas putida* plasmid pWW0 (Tark et al.,...
Expression of these genes in *E. coli* and *P. putida* responds to DNA damage (Kitagawa *et al.*, 1985; Tark *et al.*, 2005), suggesting that *umuDpR-umuC* genes from pUM505 may also respond likewise. Supporting this hypothesis, when total RNA from *P. aeruginosa* PAO1 (pUM505) cells exposed to the DNA damage-inducing agent MMC was analysed by RT-PCR, an intense band, which contains the overlap region of *umuDpR-umuC*, was detected (Fig. 2a, lane 1). To confirm this result, the putative regulatory region was cloned upstream of the promoterless lacZ reporter gene in the pLP170 vector, and β-galactosidase activity was measured. NB-grown *P. aeruginosa* PAO1 cells carrying plasmid pLP_umuDC showed significant LacZ activity (Fig. 2c, white bars), indicating that the *umuDpR* promoter is functional under non-stressing conditions. This result is in agreement with a previous report showing that pseudomonads have a higher baseline expression of particular SOS regulon genes, such as *umuDC* genes, even in the absence of DNA damage (Kivisaar, 2010). However, when cells were exposed to sub-inhibitory levels of MMC, higher β-galactosidase activity was observed (Fig. 2c, black bars); the *umuDpR* promoter increased the activity about 2.5 times with respect to the untreated control after 3 h of incubation. It can be concluded from these data that expression of the *umuDpR-umuC* operon is induced by DNA damage caused by MMC.

**Functional analysis of *umuDpR-umuC* genes**

As mentioned previously, the UmuDpR protein exhibits identity with the LexA repressors of *P. aeruginosa* and *E. coli*, as well as with the UmuDAb regulators of *A. baumannii* and *A. baylyi*. Furthermore, UmuDpR possesses residues required for self-cleavage that more closely resemble those of the LexA-type repressors than those from the UmuD proteins that constitute the Pol V enzyme. To elucidate the function of UmuDpR as a regulator of the SOS response, the *umuDpR-umuC* genes were amplified by PCR and cloned into the high-copy-number pUCP20 vector. The resulting recombinant plasmid, pUC_umuDC, was transferred into *P. aeruginosa* PAO1, and susceptibility tests to MMC were performed. *P. aeruginosa* PAO1 transformants expressing *umuDpR-umuC* genes showed increased sensitivity to MMC as compared with the control PAO1 (pUCP20) strain (Fig. 3a). This behaviour may be attributed to overproduction of UmuDpR, which represses expression of SOS genes thus diminishing the DNA damage repair effects of the SOS pathway. The small size of pUM505 UmuC*, and the fact that it lacks domains involved in DNA and protein interaction, suggested that it is not functional. Accordingly, *P. aeruginosa* PAO1 transformants expressing *umuDpR-umuC* genes showed increased sensitivity to MMC as compared with the control PAO1 (pUCP20) strain (Fig. 3a). This result confirms that UmuDpR is responsible for the higher MMC susceptibility observed. The sequence of the *umuDpRC* operon appears to have been corrupted by mutation and, although its expression continues to respond to DNA damage, the truncated UmuC* protein is not functional.

To examine whether the UmuDpR protein is able to complement the function of the LexA repressor, the pUC_umuD plasmid was transferred into strain PW6037, a *P. aeruginosa* PAO1-derived knockout mutant affected in the *lexA* gene. The identity of the *lexA* mutant was confirmed by PCR (data not shown), employing specific primers (Table S1). As expected, the PW6037 strain was more resistant to
MMC than the wild-type PAO1 strain (Fig. 3b). This result is in agreement with previous reports showing that *E. coli* mutants affected in the *lexA* gene are more resistant to DNA damaging agents than wild-type strains, because several genes, whose products participate in DNA repair, are constitutively expressed (Walker, 1984; Fernández de Henestrosa et al., 2000). However, when the PW6037 strain was transformed with the pUC_umuD plasmid, it displayed an intermediate susceptibility phenotype to MMC (Fig. 3b); this result indicates that UmuDpR is able to complement, at least partially, the function of the *P. aeruginosa* LexA protein and suggests that UmuDpR could act as a repressor of SOS response genes controlled by LexA.

**UmuDpR regulates expression of SOS genes**

Because the *P. aeruginosa* PAO1 genome lacks *umuDC* genes (Kivisaar, 2010), and the UmuDpR protein possesses characteristics of LexA-type repressors, we tested directly whether UmuDpR is able to regulate the expression of *P. aeruginosa* SOS genes. For this purpose, we selected *phl*, *imuA*, *lexA* and *recA* genes (Table S2), which are part of the SOS regulon in other bacteria; all these genes are regulated by LexA in the PAO1 strain (Cirz et al., 2006).

qRT-PCR analyses were carried out using total RNA from the WT *P. aeruginosa* PAO1 strain, the PW6037 *lexA*− mutant and the PW6037 mutant transformed with the pUC_umuD plasmid. The results showed that expression of *recA*, *imuA* and *lexA* genes was upregulated 5.3-, 4.3-, and 3.4-fold, respectively, in the *lexA*− mutant compared with expression of the correspondent genes in the WT *lexA*+ strain (Fig. 4). This behaviour was expected, given the repressor nature of the LexA protein. Interestingly, the presence of *umuDpR* in the *lexA*− mutant caused the expression of these three genes to decrease significantly, although to different levels (Fig. 4). These data confirmed that UmuDpR is a transcriptional regulator able to repress the expression of SOS genes. This transcriptional control included the genes encoding RecA and LexA, the main modulators of the SOS response, and the *imuA* gene.

**Fig. 3.** Susceptibility to MMC by bacterial strains. Cultures were grown in NB for 18 h at 37 °C with shaking with the indicated concentrations of MMC and OD_600 was measured. (a) (open circles), *P. aeruginosa* PAO1 (pUCP20); (filled squares), PAO1 (pUC_umuDC); (open squares), PAO1 (pUC_umuD); (b) (open circles), *P. aeruginosa* PAO1; (filled circles) PW6037 *lexA*− mutant; (open squares) PW6037 (pUC_umuD). Data shown are means of three assays in duplicate with se bars shown.

**Fig. 4.** Effect of UmuDpR on the expression of *P. aeruginosa* PAO1 SOS genes. Cultures were grown in NB to the mid-exponential growth phase and total RNA was isolated and processed as described in Methods. Relative expression of each gene corresponds to wild-type PAO1 (WT) (white bars), PW6037 *lexA*− mutant (black bars) or PW6037 transformed with the pUC_umuD plasmid (*lexA*−/umuDpR) (grey bars). Values represent the means of three independent determinations in duplicate with se bars shown, normalized with respect to transcription of the *rpoB* gene, calculated as described in Methods.
which has been involved in DNA-damage-induced mutagenesis in the alphaproteobacterium Caulobacter crescentus (Gallhardo et al., 2005). Transcription of the phi gene, which encodes a photolyase-like enzyme that repairs UV-induced DNA lesions, showed no significant changes in both the lexA mutant and in its umuDpR transformant (Fig. 4). Regulation of SOS genes by UmuDpR probably varies due to differences in its affinity for the regulatory regions of the distinct genes. To further analyse the role of the UmuDpR protein as a transcriptional regulator, interaction of UmuDpR protein with SOS gene operators was tested. The UmuDpR gene was first cloned into an expression vector adding a 6-His tag at the protein C-terminus and the UmuDpR-His recombinant protein was overexpressed (Fig. S1). UmuDpR-His was recovered at high purity after nickel affinity chromatography (Fig. S1) and it was used in EMSA. These assays, however, did not show any specific binding of UmuDpR to the 5′ regions of SOS genes (Fig. S2 and data not shown). The Ps. aeruginosa LiuR transcriptional factor displayed a clear retardation effect (Fig. S2), thus validating the EMSA. These data, and the fact that UmuDpR lacks the DNA-binding N-terminal domain of LexA (Fig. 1b), suggest that transcriptional regulation by UmuDpR involves an indirect mechanism. Thus, UmuDpR may require an additional cofactor(s) to accomplish its regulatory functions, as previously postulated for the homologous UmuDab protein encoded in the A. baylyi chromosome (Hare et al., 2012).

Inhibition of SOS response induction by plasmid-encoded gene products was first reported in E. coli by Bagdasarian et al. (1980, 1986); these authors identified the PsiB protein from the E. coli conjugative plasmid R100.1 as responsible for inhibition of the generation of an SOS signal. It was later shown that PsiB does not affect expression of the SOS pathway (Bagdasarian et al., 1992), but directly binds the RecA protein (Petrova et al., 2009), thus impairing its DNA-damage repair activities. To our knowledge, the UmuDpR protein represents the first report of a LexA-type transcriptional regulator that is encoded by a plasmid. As previously postulated by Bagdasarian et al. (1986, 1992) for the PsiB protein, UmuDpR may protect the plasmid conjugative transfer process, which involves the transient formation of single-stranded DNA, which may trigger the potentially deleterious SOS response. Thus, the SOS response not only involves activities to survive or to change, but also includes actions devoted to sharing information with neighbouring cells (Baharoglu & Mazel, 2014). Possession of an SOS response repressor could be evolutionarily advantageous for the conjugative pUM505 plasmid.

In summary, our results indicate that UmuDpR from pUM505 participates in regulation of the expression of SOS genes by an indirect mechanism, and suggest that the functioning of this protein as an anti-SOS effector can be an evolutionary strategy to maintain the integrity of the plasmid and to adapt it to new bacterial hosts.

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