**DnaA and LexA Proteins Regulate Transcription of the uvrB Gene in Escherichia coli: The Role of DnaA in the Control of the SOS Regulon**

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The uvrB gene belongs to the SOS network, encoding a key component of the nucleotide excision repair. The uvrB promoter region contains three identified promoters with four LexA binding sites, one consensus and six potential DnaA binding sites. A more than threefold increase in transcription of the chromosomal uvrB gene is observed in both the ΔlexA ΔsulA cells and dnaA<sub>A345S</sub> cells, and a fivefold increase in the ΔlexA ΔsulA dnaA<sub>A345S</sub> cells relative to the wild-type cells. The full activity of the uvrB promoter region requires both the uvrB<sub>p1-2</sub> and uvrB<sub>p3</sub> promoters and is repressed by both the DnaA and LexA proteins. LexA binds tightly to LexA-box1 at the uvrB<sub>p1-2</sub> promoter irrespective of the presence of DnaA and this binding is important for the control of the uvrB<sub>p1-2</sub> promoter. DnaA and LexA, however, compete for binding to and regulation of the uvrB<sub>p3</sub> promoter in which the DnaA-box6 overlaps with LexA-box4. The transcription control of uvrB<sub>p3</sub> largely depends on DnaA-box6. Transcription of other SOS regulon genes, such as recN and dinJ, is also repressed by both DnaA and LexA. Interestingly, the absence of LexA in the presence of the dnaA<sub>A345S</sub> mutant leads to production of elongated cells with incomplete replication, aberrant nucleoids and slow growth. We propose that DnaA is a modulator for maintenance of genome integrity during the SOS response by limiting the expression of the SOS regulon.

**Keywords:** DnaA, LexA, uvrB gene expression, SOS regulon, regulation

**INTRODUCTION**

The uvrB gene encodes the UvrB protein, one of the key components of the NER system (Truglio et al., 2006). NER repair is a versatile pathway that recognizes a wide range of DNA lesions by the concerted function of the UvrABC proteins (Pruteanu and Baker, 2009). The uvrB gene belongs to the SOS regulon (Howard-Flanders et al., 1966). SOS is a global response to DNA damage in which RecA filaments bound on ssDNA promote self-cleavage of the LexA protein. Cleavage of LexA induces expression of the SOS genes, resulting in an arrest of cell division for the time required to repair the damages (Walter, 1996). LexA regulates the...
SOS regulon by binding to the LexA-box and thus preventing gene expression during normal growth. The LexA-box has the following consensus sequence TACTG(TA)₅CACGTA (Walker, 1984), having a conserved trimer of CTG on the left and another trimer of CAG on the right with a variable sequence of spacers; the spacing between “CTG” and “CAG” is 10 nucleotides (Fernandez De Henestrosa et al., 2000; Wade et al., 2005). LexA contains two domains: an N-terminal winged helix-turn-helix (WHH) DNA-binding domain and a C-terminal dimer with a latent protease domain (Zhang et al., 2010). In response to DNA damage RecA-ssDNA-ATP filaments are formed and the auto-proteolytic activity of LexA at the C-terminal domain is activated by interacting with the filaments. The degradation of LexA opens the promoter region for RNA polymerase (RNAP) recruitment and the start of transcription.

The DnaA protein initiates chromosomal replication in bacteria by interacting with 9-mer consensus sequences of TTA/TTNCACA, the DnaA-boxes, at the origin for replication (Kornberg and Baker, 1992; Schaper and Messer, 1995). DnaA has a high affinity for ATP and ADP (Sekimizu et al., 1987), and ATP-DnaA is active for the initiation of replication whereas ADP-DnaA is inactive (Sekimizu et al., 1987). DnaA is also a transcription factor, repressing transcription by binding to DnaA-boxes in the promoter regions, such as those found at the promoters for the dnaA gene itself (Atlung et al., 1985; Braun et al., 1985), the mioC gene (Lother et al., 1985) and the nrd operon (Tuggle and Fuchs, 1986; Speck et al., 1999; Olliver et al., 2010) while transcription of the polA gene is stimulated by DnaA in stationary phase (Quinones et al., 1997).

The E. coli uvrB promoter region contains three promoters, namely, uvrBp1, uvrBp2, and uvrBp3 (Sancar et al., 1982). A LexA-box with the AACTGTTTTTTATCCAGTA sequence has been identified between the -35 and -10 regions of uvrBp2 (Fernandez De Henestrosa et al., 2000). Interestingly, the uvrBp3 promoter contains DnaA boxes (Arikan et al. 1986) which constitute the DARS1 (DnaA reactivation site) site consisting of three DnaA-boxes, where inactive ADP-DnaA is reactivated to form active ATP-DnaA (Fujimitsu et al., 2009). Here, we show that the uvrB promoters are repressed by both DnaA and LexA by specifically binding to its promoter region in either a competitive or an independent manner. Interestingly, two other genes of the SOS regulon, recN and dinJ, are also found to be repressed by both DnaA and LexA. The simultaneous absence of LexA- and DnaA-dependent repression leads to production of elongated cells with incomplete DNA replication with abnormal nucleoids and slow growth. It is likely that regulation of gene expression by DnaA maintains genome integrity during the SOS response in Escherichia coli.

**MATERIALS AND METHODS**

**Bacterial Strains**

All bacterial strains used in this study are derived from the *E. coli* K12 listed in Table 1. The ΔsulA::neo allele was transferred into the MC4100 by P1 transduction (Miller et al., 1992) and resulting in MC4100ΔsulA::neo. The cat gene was PCR amplified using the plasmid pKD3 as template and primer 582 and 583 as listed in Supplementary Table S2 and inserted into MC4100ΔsulA::neo mutant to replace the chromosomal lexA gene through homologous recombination by One-step Chromosomal Gene Inactivation method (Datsenko and Wanner, 2000), resulting in MC4100ΔsulA::neoΔlexA::cat double mutant. The neo or cat genes were removed by the FRT site-specific recombination as described previously (Datsenko and Wanner, 2000), resulting in MC4100ΔsulA or/and MC4100ΔsulAΔlexA double mutant. The cat gene was also PCR amplified using pKD3 as template and primers 48 and 49 listed in Supplementary Table S2, then inserted behind the chromosomal uvrB gene in MC4100 and MC4100ΔsulAΔlexA cells by the method mentioned above. The cat gene was replaced by pCE36 using the FRT site-specific recombination in the cells mentioned, resulting in insertion of the lacZ::neo fusion behind the chromosomal uvrB gene as described previously (Datsenko and Wanner, 2000; Ellermeier et al., 2002). As a result, MC4100uvrB-lacZ::neo, MC4100ΔsulA uvrB-lacZ::neo and MC4100ΔsulAΔlexA uvrB-lacZ::neo cells were constructed. The dnaAΔ345S::cat allele was transferred into the MC4100uvrB-lacZ::neo, MC4100ΔsulA uvrB-lacZ::neo and MC4100ΔsulAΔlexA uvrB-lacZ::neo cells by P1 transduction (Miller et al., 1992). The lexAΔ..tet allele was P1 transduced into MC4100 and dnaAΔ345S::cat cells carrying uvrB-lacZ::neo fusion, respectively (Miller et al., 1992). For construction of recN-lacZ::neo and dinJ-lacZ::neo, the cat gene was PCR amplified using the plasmid pKD3 as template and primers 1229 and 1230 for recN-lacZ::neo, primes 1235 and 1236 for dinJ-lacZ::neo as listed in Supplementary Table S2. After insertion of the cat gene down-stream of the chromosomal recN or dinJ gene in MC4100 cells, the cat gene was replaced by pCE36 using the FRT site-specific recombination, resulting in insertion of the lacZ::neo fusion behind the chromosomal recN or dinJ gene as described previously (Datsenko and Wanner, 2000; Ellermeier et al., 2002). The recN-lacZ::neo or dinJ-lacZ::neo allele was P1 transduced into dnaAΔ345S, ΔsulAΔlexA, ΔsulAΔlexA dnaAΔ345S and lexA3 dnaAΔ345S cells, resulting in dnaAΔ345S recN-lacZ::neo, ΔsulAΔlexA recN-lacZ::neo, ΔsulAΔlexA dnaAΔ345S recN-lacZ::neo, lexA3 dnaAΔ345S recN-lacZ::neo, or dnaAΔ345S dinJ-lacZ::neo, ΔsulAΔlexA dnaAΔ345S dinJ-lacZ::neo and lexA3 dnaAΔ345S dinJ-lacZ::neo. DH5α was used as a host for the preparation of plasmid DNA. The WM2287 strain containing the pdnaA116 plasmid was used for DnaA purification (Schaper and Messer, 1995) and BL21-Gold (DE3) for His₆-LexA protein expression and purification.

**Growth Media and Conditions**

Cells were grown at 37°C in LB (Bertani, 1951) or ABTGcasa medium (Morigen et al., 2005). When necessary, 100 µg/ml of ampicillin, 30 µg/ml of chloramphenicol, 15 µg/ml of tetracycline and 50 µg/ml of kanamycin were added.

**Plasmid Constructions**

All plasmids used in this study are listed in Table 2 and the primers including their descriptions are listed in
Supplementary Table S2. The uvrBp1-3 promoter was PCR amplified using chromosomal DNA from the wild-type BW25113 cells as template and primers 54 and 57. The uvrBp1-3 PCR fragment was inserted in front of the promoterless lacZ gene on pTAC3953 (Brondsted and Atlung, 1994) at BamHI and HindIII sites, resulting in MiniR1−uvrBp1-3-lacZ. Using the same template, the uvrBp1-2 promoter region was amplified by primers 79 and 57, and the uvrBp3 promoter region by primers 54 and 71. The PCR fragment for each promoter was then inserted into pTAC3953 at the BamHI and HindIII sites (Brondsted and Atlung, 1994), leading to the construction of plasmids puvrBp1-2-lacZ and puvrBp3-lacZ. The uvrBp1-3-lacZ fusion was PCR amplified by primers 54 and 1131 using puvrBp1-3-lacZ as template. The PCR fragment was then inserted into a low copy plasmid, MiniR1 which is about 1–2 copies per chromosome per ter site (Morigen et al., 2001) at the BamHI and HindIII sites, resulting in MiniR1−uvrBp1-3-lacZ (shown as R1-uvrBp1-3 for short). The uvrBp1-2-lacZ fusion was PCR amplified by primers 79 and 1350 using puvrBp1-2-lacZ as template. The PCR fragment was then inserted into MiniR1 at the BamHI and BglII sites, resulting in MiniR1−uvrBp3-lacZ (R1-uvrBp3 for short). The DH5α cells were transformed with the resulting ligation. The lexA gene was PCR amplified using the chromosomal DNA from the wild-type BW25113 cells as template and primers 57 and 58. The PCR fragment for lexA was inserted into pET28a (EMD Biosciences) at the NcoI and XhoI sites, resulting in pET28a-lexA which produces His6-LexA protein fusion under IPTG induction. All constructions were sequenced to make sure the plasmid constructions were correct.

### Site-Directed Mutagenesis

Point mutation was generated using a site-directed mutagenesis kit (TransGen Biotech, Beijing, China) as described previously (Rousseau et al., 2013). The mutations (from TG to CA) in DnaA-box1 in the uvrBp1-2 promoter were generated by site-directed mutagenesis using the puvrBp1-2-lacZ plasmid as template and the pair of primers 1214 and 1215. Similarly, using the puvrBp3 plasmid as template, the mutations (from TG to CA) in DnaA-box2 in uvrBp3 promoter, were generated by the pair of primers 1210 and 1211.

### Purification of Proteins

The DnaA protein was over-expressed in WM2287/pdnaA116 plasmid constructions were correct.

| Strain | Genotype | Source |
|--------|----------|--------|
| MC4100 | Wild type F− araD194 Δ (lac) U169 strA thi | Casadasan, 1976; Ferenci et al., 2009 |
| BW25113 | Wild type mB3 ΔlacZ4787 hasdR514 Δ(araBAD)567 Δ(rhaBAD)588 rph−1 | Baba et al., 2006 |
| JWO041-KC | BW25113 ΔsuA−:neo | Baba et al., 2006 |
| SMG379 | MG1655 dnaAs3565...−MinTn10 cat | Gon et al., 2006 |
| JC13199 | lexA3malE...−Tn10 | Clark, 1973 |
| MOR741 | MC4100 uvrB-lacZ...neo | This work |
| MOR746 | MC4100 dnaAs3565...−cat uvrB-lacZ...neo | This work |
| MOR2395 | MC4100 ΔsuA ΔlexA dnaAs3565...−cat uvrB-lacZ...neo | This work |
| MOR2399 | MC4100 ΔsuA ΔlexA dnaAs3565...−cat uvrB-lacZ...neo | This work |
| MOR2670 | MC4100 ΔsuA:neo | This work |
| MOR2672 | MC4100 ΔsuA uvrB-lacZ...neo | This work |
| MOR798 | MC4100 lexA3...−tet uvrB-lacZ...neo | This work |
| MOR803 | MC4100 lexA3...−tet dnaAs3565...−cat uvrB-lacZ...neo | This work |
| MOR749 | MC4100 dnaAs3565...−cat | This work |
| MOR1466 | MC4100 ΔsuA ΔlexA | This work |
| MOR1511 | MC4100 ΔsuA ΔlexA dnaAs3565...−cat | This work |
| MOR2395 | MC4100 recN-lacZ...neo | This work |
| MOR2586 | MC4100 dnaAs3565...−cat recN-lacZ...neo | This work |
| MOR2587 | MC4100 ΔsuA ΔlexA recN-lacZ...neo | This work |
| MOR2588 | MC4100 ΔsuA ΔlexA dnaAs3565...−cat recN-lacZ...neo | This work |
| MOR2589 | MC4100 lexA3...−tet dnaAs3565...−cat recN-lacZ...neo | This work |
| MOR2590 | MC4100 dinJ-lacZ...neo | This work |
| MOR2591 | MC4100 dnaAs3565...−cat dinJ-lacZ...neo | This work |
| MOR2592 | MC4100 ΔsuA ΔlexA dinJ-lacZ...neo | This work |
| MOR2593 | MC4100 ΔsuA ΔlexA dnaAs3565...−cat dinJ-lacZ...neo | This work |
| MOR2594 | MC4100 lexA3...−tet dnaAs3565...−cat dinJ-lacZ...neo | This work |
| BL21-Gold (DE3) | E. coli B F− ompT hsdSB (rB− mQ−) dcm− T7el (DE3) endA Hte | Agilent Technologies |
| DH5α | F− supE44 ΔlacU169(ΔlacZΔM15) hsdR17 recA1 endA1 gyrA96 | New England Biolabs |
(Olliver et al., 2010). The BL21-Gold (DE3)/pET28a-his6-LexA cells were grown at 37°C in 200 ml of LB medium. At OD600 = 0.6, IPTG with 0.3 mM of final concentration was added and incubated for 2 h. The cells were harvested and washed with PBS once, resuspended in 10 ml of the lysis buffer (50 mM NaH2PO4, 300 mM NaCl, 10 mM imidazole), and sonicated. The whole cell lysate was mixed with His-Select Ni-NTA slurry (Qiagen) and His6 was purified according to the manufacturer instructions. Purity of the His6-LexA protein sample was detected by staining with INSTANT BLUE (Expeodeon) after SDS-PAGE (Supplementary Figure S1) gel-electrophoresis following the manufacturer instructions. The His6-LexA protein concentration was determined by BCA assay (Thermo Scientific) and stored at −80°C after imidazole was removed by dialysis in 1xPBS buffer (0.137 M NaCl, 0.027 M KCl, 0.01 M Na2HPO4, 0.0018 M KH2PO4).

**β-Galactosidase Activity Assay**

Exponentially growing cells (1 ml) at 37°C in ABTGcasa medium were collected at OD450 = 0.1, 0.2, 0.3, 0.4, and 0.5, then mixed with cold toluene (0.1 ml) and kept on ice immediately. For measurement of β-galactosidase activity, 0.2 ml tolune-treated sample was added to 1 ml Z buffer (40 mM NaH2PO4, 60 mM Na2HPO4, 10 mM KCl, 1 mM MgSO4 and 50 mM β-mercaptoethanol, pH 7.0) containing 0.66 mg/ml o-nitrophenyl-β-D-galactopyranoside. The reaction was performed at 30°C until the color changed to yellow and stopped by addition of 0.5 ml 1 M Na2CO3, and the absorbance at OD420 was measured. The β-galactosidase activity was calculated by 1000°OD420/reaction time (min) * OD450 * 0.2 ml (Miller, 1972).

### DNase I Footprinting Assays

The uvrB promoter region (523 bp) was PCR amplified using chromosomal DNA from the wild-type BW25113 cells as template and primer 828 and 829 for the DNase I footprinting assays. In the PCR reaction, the primer 828 was 5′ labeled with [γ-32P]ATP (GE Healthcare) by T4 polynucleotide kinase (New England Biolabs). The PCR product was purified with the Bio-Spin 6 Columns (Bio-Rad) according to the manufacturer instructions. Approximately 1 nmol of labeled DNA and increasing amounts (final concentration was 50, 100, 200, 300, and 600 nM) of ATP-DnaA or His6-LexA protein were mixed in a 10 µl reaction buffer containing 1 mM DTT, 0.5 mg/ml Ac-BSA, 20 mM HEPES pH 7.6, 50 mM K-glu, 5 mM MgCl2 and 3 mM of ATP (Sigma-Aldrich). The reaction mixture was incubated at 37°C for 15 min. Then, 4 mg/ml of DNase I diluted in digestion buffer (25 mM Tris pH 7.5, 5 mM MgCl2, 1 mM CaCl2, 2 mM DTT and 100 mM KCl) was added and the mixture was incubated at 37°C for 20 s. To determine the protection patterns of the uvrB promoter DNA by two proteins (DnaA and LexA), the second protein was added with final concentrations of 50, 100, 200, 300, and 600 nM after 10 min incubation with the first protein with final concentration of 200 nM, and incubated for 10 min, then digested by DNase I for 30 s. The DNase I digestion was stopped by addition of an equal volume of formamide loading buffer (90% formamide, 1 × TBE, bromophenol blue, xylene cyanol, calf thymus non-specific DNA). Samples were incubated for 5 min at 95°C and analyzed by 6% acrylamide in denaturing conditions (8 M urea and 1 × TBE buffer) by comparison with a DNA sequence ladder generated with the same primers using a A + G reaction as described previously (Maxam and Gilbert, 1980). After electrophoresis, gels were dried and autoradiographed.

### TABLE 2 | Plasmids.

| Plasmids | Description | Source |
|----------|-------------|--------|
| pKD3 | repKEx, bla FRT cat FRT | Datsenko and Wanner, 2000 |
| pKD4 | repKEx, bla FRT neo FRT | Datsenko and Wanner, 2000 |
| pKD46 | repK25C101, bla PAM240 γβ exo | Datsenko and Wanner, 2000 |
| pCP20 | repK25C101, bla cat cR57pR | Datsenko and Wanner, 2000 |
| pCE36 | repKEx neo FRT lacZY tR2 | Eilersmeier et al., 2002 |
| pET-28a | repK25C111 neo lacZ pT7 | EMD Biosciences |
| pET28a-his6-lexA | The lexA gene was inserted into pET28a at Ncol and Xhol to produce His6-LexA fusion. | This work |
| pCDS116 | repK25C111 bla lacI P2A_{–3}–93 dnaA tet93A | Krause et al., 1997 |
| pTAC3953 | repK25C111 neo lacZ | Brondsted and Atlung, 1994 |
| puvBp1-3-lacZ | The whole cluster of the uvrB promoters including uvrBp1, 2, and 3 was inserted in front of the lacZ gene on pTAC3953 at the BamHI and HindIII sites. | This work |
| puvBp1-2-lacZ | The uvrBp1-2 fragment was inserted in front of the lacZ gene on pTAC3953 at the BamHI and HindIII sites. | This work |
| puvBp3-lacZ | The uvrBp3 fragment was inserted in front of the lacZ gene on pTAC3953 at the BamHI and HindIII sites. | This work |
| puvBp3Δ279-172-lacZ | A fragment from −279 to −172 was deleted from the plasmid puvBp1-3-lacZ. | This work |
| pluvBp1-3-lacZ | The uvrBp1-3-lacZ fusion fragment was inserted at the BamHI and HindIII sites onto MiniR1. | This work |
| MiniR1 | R1 derived vector, containing oriR1, bla, repA, tap, cspA | Morigen et al., 2001 |
| MiniR1-uvrBp1-3-lacZ | The uvrBp1-3-lacZ fusion fragment was inserted at the BamHI and HindIII sites onto MiniR1. | This work |
| MiniR1-uvrBp1-2-lacZ | The uvrBp1-2-lacZ fragment was inserted at the BamHI and BglII sites onto MiniR1. | This work |
| MiniR1-uvrBp3-lacZ | The uvrBp3-lacZ fragment was inserted at the BamHI and BglII sites onto MiniR1. | This work |
| puvBp1-2GC-lacZ | TG in LexA-box1 was replaced by GC on puvBp1-2-lacZ. | This work |
| puvBp3CA-lacZ | TG in DNA-box6 was replaced by CA on puvBp3-lacZ. | This work |
| puvBp3CA-lacZ | TG in DNA-box6 was replaced by CA on puvBp3-lacZ. | This work |
UV Irradiation
Cells were exponentially grown at 37°C in 50 ml of ABTGcasa medium, 20 ml of cell culture at OD<sub>600</sub> = 0.08 was irradiated in an open petri-dish with 50 J/m² of UV, then cells were grown in flask at 37°C. Sampling and measurement of β-galactosidase activity was carried out as mentioned above.

Flow Cytometry
Exponentially growing cells in ABTGcasa medium at 37°C were treated with 300 μg/ml rifampicin and 10 μg/ml cephalexin for 4–5 generations. Initiation of DNA replication is inhibited by rifampicin which allows ongoing replication finish while cell division is blocked by cephalexin at the time of addition of the drugs (Skarstad et al., 1986; Boye and Lobner-Olesen, 1991). Cells were fixed in 70% ethanol and stained in Hoechst 33258 (Invitrogen) for 30 min, then analyzed by flow cytometry (LSR Fortessa, BD).

Confocal Fluorescence Microscopy
Exponentially growing cells in ABTGcasa medium at 37°C were harvested, fixed in 70% ethanol, visualized under a Zeiss LSM710 Confocal microscope with 100×/1.4 Plam-Apo at 405 nm laser excitation after staining in Hoechst 33258 for 30 min. Images were scanned by a PMP detector and analyzed with the ZEN 2011 (black version) software to measure cell size and nucleoid distribution.

RESULTS
Both DnaA and LexA Repress Expression of the uvrB Gene
A global transcriptional analysis by using Affymetrix GeneChip E. coli Genome 2.0 arrays showed that expression of the uvrB gene increased 2.7 (±0.9)-fold in a dnaA<sub>A34SS</sub> mutant relative to the wild-type cells (Morigen and Skarstad, unpublished data). The result suggests that DnaA could directly be involved in control of the uvrB gene expression since DnaA<sub>A34SS</sub> binds to DnaA-boxes with a lower affinity compared to wild-type DnaA. The dnaA<sub>A34SS</sub> mutant is a suppressor for a mutant lacking four of the redoxins involved in Nrd activity (Ortenberg et al., 2004) and the purified DnaA<sub>A34SS</sub> protein is defective for ATP binding <i>in vitro</i> (Gon et al., 2006). The dnaA<sub>A34SS</sub> mutant is also found to result in under-replication and larger cell mass with slower growth (Ortenberg et al., 2004; Gon et al., 2006). In order to determine the regulatory effect of DnaA on transcription of the uvrB gene, the lacZ reporter gene was inserted downstream of the chromosomal uvrB gene, resulting in an <i>uvrB-lacZ</i> derivative of the MC4100 strain lacking the chromosomal lacZ gene (Casadaban, 1976; Ferenci et al., 2009). Subsequently, a dnaA<sub>A34SS</sub> allele was transferred to the <i>uvrB-lacZ</i> strain by P1 transduction. The uvrB gene belongs to the SOS regulon which is regulated by LexA (Howard-Flanders et al., 1966). It should be noted that the LexA protein is essential for cell growth but the growth defect in the absence of LexA can be suppressed by deletion of the sulA gene (George et al., 1975). Thus, we removed the lexA gene by constructing the ΔlexA ΔsulA double mutant. To understand how DnaA interacts with LexA in the control of uvrB expression, the <i>uvrB-lacZ</i> allele was P1 transduced into the ΔlexA ΔsulA, ΔlexA ΔsulA <i>dnaA<sub>A34SS</sub></i>, lexA3 and lexA3 <i>dnaA<sub>A34SS</sub></i> cells including the ΔsulA mutant as a control. The level of transcription from the uvrB promoter region was measured by the β-galactosidase activity assay in exponentially growing cells. Transcription from the uvrB promoter was 3.5-fold higher in the <i>dnaA<sub>A34SS</sub></i> cells compared with that of the wild-type cells (Figure 1A), suggesting that DnaA represses uvrB expression. Not surprisingly, transcription from the uvrB promoter region in the ΔsulA mutant was about the same as that in the wild-type cells, while it was 3.3-fold higher in the ΔlexAΔsulA mutant compared to the control, in agreement with previous work (Sancar et al., 1982), indicating that LexA represses uvrB expression. Interestingly, uvrB transcription was further increased, to 5.2-fold, in the ΔlexAΔsulA <i> dnaA<sub>A34SS</sub></i> triple mutant (Figure 1A), implying that the repression by DnaA and LexA of uvrB expression is additive and thus might be independent of an interaction between the two proteins. The conclusion is supported by 1.6-fold increase of uvrB expression in the ΔsulAΔlexA <i> dnaA<sub>A34SS</sub></i> cells compared to that in the ΔsulAΔlexA cells (Figure 1A inset). The LexA3 mutant protein is not self-cleavable or largely resistant to cleavage and thus binds to the uvrB promoter region regardless of whether the SOS response is on or off (Little et al., 1980; Markham et al., 1981). Transcription from the uvrB promoter region in the lexA3 mutant was about the same as that in the wild-type cells, while it was 3.6-fold higher in the lexA3 <i> dnaA<sub>A34SS</sub></i> strain (Figure 1B). These results support the idea that DnaA-dependent repression of uvrB expression is independent of LexA activity. We conclude that in the absence of the SOS response both DnaA and LexA repress transcription of the uvrB gene and function independently of each other.

The uvrB Promoter Region Contains Seven Potential DnaA-Boxes and Four LexA-Boxes
In order to understand how LexA and DnaA function in the control of uvrB transcription, we searched for the sequences corresponding to DnaA-boxes and LexA-boxes in the uvrB promoter region. The uvrB promoter region was previously shown to contain three DnaA-boxes (Fujimitsu et al., 2009) and one LexA-box (Van Den Berg et al., 1985). By our analysis, four additional DnaA-boxes and three potential LexA-boxes were identified in the region (Figure 2). All these DNA-boxes were renamed as DNA-box1, 2, 3, 4, 5, 6, and 7 from the proximal to the distal site relative to the transcription start site of the uvrBp1 promoter (Figure 4A). The characterized LexA-boxes were called LexA-box1, 2, 3, and 4, also in the same orientation (Figure 2). LexA-box1 is closest to the consensus, having the conserved CTG trimer on the left and the CAG trimer on the right end, with nine ATs out of ten bases between these two trimers. LexA-box2, 3, and 4, however, have a CTG on the left but do not have CAG on the right end, having an AT-rich sequence in the middle. LexA-box1 is located between the −35 and −10 sites of the uvrBp2
DnaA Interferes With Binding of LexA to LexA-Box2 and 3 but Not to LexA-Box1

As described above, we have shown that both DnaA and LexA repress transcription from the uvrB promoter cluster, which contains seven potential DnaA-boxes and four LexA-boxes. Now the questions are: (i) do DnaA and/or LexA bind to these potential DnaA-boxes and/or LexA-boxes? (ii) do DnaA and LexA compete for their binding sites in the uvrB promoter region? To address these questions, we performed in vitro DNase I footprinting experiments and determined the binding patterns of DnaA and LexA to the uvrB promoter cluster. A PCR amplified fragment (523 bp) of the uvrB promoter cluster was used in these experiments. The DnaA protein was purified as described previously (Olliver et al., 2010) and His6-LexA was purified as described in Materials and Methods (Supplementary Figure S1).

A protection pattern of the uvrB promoter cluster by increasing concentrations of LexA was detected in the presence or absence of DnaA. As shown in Figure 3, LexA protections of LexA-box2, 3, and 4 increased as a function of its concentration whereas LexA-box1 became protected at the lowest concentration of LexA. These results are in agreement with the differences in the LexA-box2, 3, and 4 sequences relative to the consensus sequence of LexA-box1. In the presence of DnaA, the LexA protections of LexA-box2, 3, and 4 were weakened or abolished in a DnaA concentration dependent manner while binding to LexA-box1 remained strong (Figure 3). For the DARS site, which has the LexA-box4 overlapping with DnaA-box5 and 6, the LexA protection was clear. While the overlap of the protection of the two proteins at the DARS site makes it difficult to determine whether LexA is still bound in the presence of DnaA, the appearance of the DnaA protections at the same concentration as in the absence of LexA suggests that the former can bind even in the presence of the latter and could thus displace it (Figure 3). These results suggest that LexA binds to LexA-box1 with high affinity even in the presence of high concentrations of DnaA but not to LexA-box2, 3, and 4. High concentrations of DnaA weaken the binding of LexA to its low affinity boxes. The competition of DnaA for its binding sites with LexA is not necessarily dependent on the fact that DnaA-boxes overlap with the LexA-boxes, possibly due to the ability of ATP-DnaA to form oligomeric structures.

Strong protections at DARS1 were found for ATP-DnaA (Figure 3). The DnaA protections of these sites were...
concentration dependent and such protections were not found to be changed in the presence of LexA. To further understand how DnaA and LexA function in the control of uvrB gene expression, we investigated the interaction between DnaA and LexA. The bacterial two-hybrid analysis showed that DnaA did not interact directly with LexA (data not shown).

The Full Activity of the uvrB Promoter Region Requires Both the uvrBp1-2 and uvrBp3 Promoters

The uvrB promoter region has three characterized promoters, namely uvrBp1, uvrBp2, uvrBp3 (Sancar et al., 1982), forming a cluster of uvrB promoters (Figure 4A). To determine the roles of these different promoters in the transcription control of the uvrB gene, each promoter was inserted in front of the lacZ gene into the MiniR1 plasmid. MiniR1 is a low copy plasmid, having 1–2 copies per the chromosomal ter site (Morigen et al., 2001). The resultant plasmids carry a uvrBp1-3-lacZ (for short as R1-uvrBp1-3), uvrBp1-2-lacZ (R1-uvrBp1-2) or uvrBp3-lacZ (R1-uvrBp3) fusion as illustrated in Figure 4A. The R1-uvrBp1-3 construct includes all three promoters. Each plasmid was introduced into the wild-type MC4100 cells or the dnaA A345S, 1 lexA 1 sulA or 1 lexA 1 sulA dnaA A345S derivatives (Figure 4). Promoter activity was then measured by the β-galactosidase activity assay in exponentially growing cells. Transcription from uvrBp1-2 accounted for 30% of the activity of the full-length promoter region while uvrBp3 accounted for 20% (Figure 4B). The results suggest that full activity of the uvrB promoter requires both the uvrBp1-2 and uvrBp3 promoters.

Transcription From the Plasmid-Borne uvrBp1-2 or uvrBp3 Is Repressed by DnaA and LexA Independently

To further clarify the function of DnaA and LexA in the control of the uvrBp1-2 or uvrBp3 promoter activity, transcription from the plasmid-borne uvrBp1-2-lacZ construct was measured in the dnaA A345S, ΔsulA ΔlexA and ΔsulA ΔlexA dnaA A345S mutant strains as described above. As shown in Figure 4C, transcription in the dnaA A345S or ΔsulA ΔlexA strains was about twofold higher relative to that in the wild-type cells. The transcription level further increased to 3.6-fold of wild type in the ΔlexA ΔsulA dnaA A345S cells. The increase in transcription from uvrBp1-2 in the ΔsulA ΔlexA dnaA A345S cells compared to that in the ΔsulA ΔlexA cells was clear (Figure 4C inset). These results
suggest that uvrBp1-2 promoter activity is tightly regulated by both LexA and DnaA, consistent with the presence of LexA- and DnaA-boxes in the promoters. Similarly, transcription from the plasmid-borne uvrBp3 was twofold higher in the dnaA345S or ΔsulAΔlexA cells compared with that in the wild-type cells, and a slight further increase was also found in the ΔlexAΔsulA dnaA345S mutant (Figure 4D) although the increase was not significant (Figure 4D inset). The results indicate that both DnaA and LexA repress the uvrB expression and function independently.

Repression of the uvrBp3 Promoter Is Largely Dependent on the DnaA-Box6

The footprinting analysis showed that both DnaA and LexA bound to DnaA-box5, 6, 7 and LexA-box4 of the uvrBp3 promoter. To determine the role of such binding sites in the control of the uvrBp3 promoter, we mutated the TG in DnaA-box6 to CA on the uvrBp3 plasmid (a derivative of pTAC3953 as described in section “Materials and Methods”) by site-directed mutagenesis, leading to the uvrBp3CA plasmid (Figure 5A). The mutations also changed the LexA-box1 since DnaA-box6 overlaps with LexA-box4 (Figure 5A) and destroyed the DARS1 site where ATP-DnaA is formed (Fujimitsu et al., 2009). It was found that transcription from uvrBp3CA was 5.9-fold higher relative to that from uvrBp3 in the wild-type cells (Figure 5B). Compared with uvrBp3, transcription from uvrBp3CA in the dnaA345S cells was 6.3-fold greater (Figure 5B), indicating that mutation of the site can still influence transcription in the absence of full DnaA activity, probably by influencing LexA binding. In the ΔlexAΔsulA cells, transcription from uvrBp3CA was 13.4-fold higher relative to the activity of uvrBp3 (Figure 5B). Clearly, in the absence of LexA the mutation of the DnaA-box6 results in a dramatic change in transcription, suggesting that strong repression by the wild type ATP-DnaA is decreased due to the mutations. These same mutations can also impair ATP-DnaA regeneration activity at the DARS1, leading to a decrease in accumulation of ATP-DnaA compared with the wild type plasmid (Fujimitsu et al., 2009).

LexA-Box1 Is Important for Control of uvrBp1-2 Transcription

The uvrBp1-2 promoter contains LexA-box1 and DnaA-box1. LexA-box1 remains strongly protected by LexA in the presence of high concentrations of DnaA. To understand the role of LexA-box1 in the regulation of uvrBp1-2 transcription, we mutated TG to GC in LexA-box1, resulting in plasmid uvrBp1-2GC
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FIGURE 4 | The full activity of the uvrB promoter requires the uvrBp1-2 and uvrBp3 promoters. (A) Illustration for insertion of individual uvrB promoter in front of the lacZ gene into MiniR1 plasmid. Construction of the MiniR1-uvrBp1-3-lacZ (R1-uvrBp1-3), MiniR1-uvrBp1-2-lacZ (R1-uvrBp1-2) or MiniR1-uvrBp3-lacZ (R1-uvrBp3) plasmid was as mentioned in section "Materials and Methods." Promoter uvrBp1-2 contains promoter 1 and 2, ranging from −77 to +33; promoter uvrBp3 starts from −567 and ends at −280; Promoter uvrBp1-3 consists of promoter 1, 2, and 3, including the region from −567 to +33, the whole cluster of the uvrB promoters. The nucleotide positions are as indicated in Figure 2. The open rectangles represent LexA-boxes, the open triangles represent DnaA-boxes with orientation, the hatched rectangles represent LexA-boxes overlapping with DnaA-box. The filled arrows indicate positions of the promoters and orientation of transcriptions. The p1, p2, and p3 represent the uvrB promoter 1, 2, and 3. The wild-type, dnaA<sup>A345S</sup>, ΔsulA ΔlexA and ΔsulA ΔlexA dnaA<sup>A345S</sup> cells were transformed by plasmid R1-uvrBp1-3, R1-uvrBp1-2, and R1-uvrBp3, respectively. The resultant transformants were exponentially grown in ABTGcasa medium at 37°C. Activity of the individual plasmid-borne uvrB promoter was measured as the β-galactosidase activity in the cells by Miller method (Miller, 1972). Activity of individual promoter relative to that of R1-uvrBp1-3 (229 U) in the wild-type cells is illustrated (B). Relative activity of promoter R1-uvrBp1-2 (C), or R1-uvrBp3 (D) in the dnaA<sup>A345S</sup>, ΔsulA ΔlexA and ΔsulA ΔlexA dnaA<sup>A345S</sup> cells compared with that in the wild-type cells (70 U for uvrBp1-2 and 44 U for R1-uvrBp3) is illustrated. The insets indicate the relative activity of promoters in the ΔsulA ΔlexA dnaA<sup>A345S</sup> cells against that in the ΔsulA ΔlexA cells. The values shown at top of the bars are the average of three individual experiments, and the standard errors are shown. ***Stands for P-value ≤ 0.01, ** for 0.01 < P-value ≤ 0.05, and ns represents P-value > 0.05.
and of the LexA-box1 mutation. This is expected in the case of the mutant strains is thus the same in the presence or absence of the LexA-box1 mutation. This is expected in the case of the ΔlexAΔsulA strain, however, it is surprising in the dnaA3455, ΔlexAΔsulA and ΔlexAΔsulA dnaA3455 cells since the LexA-box1 mutation should not influence binding by DnaA and a further increase in expression would be expected when DnaA is mutated. It is thus possible that this change in DNA sequence might also affect DnaA oligomerization or RNAP binding.

**UV Irradiation Increases Transcription of the uvrB Gene**

We found that transcription of the chromosomal uvrB gene was increased 3.2-fold in the uvrB-lacZ strain after UV irradiation (Figure 6A) in the wild-type cells. The level of expression did not significantly change in the ΔlexAΔsulA cells after UV irradiation (Figure 6A). These results confirm that the uvrB gene is one of the SOS genes which are regulated by the LexA protein (Howard-Flanders et al., 1966), responding to UV-induced DNA damage. Interestingly, uvrB gene transcription increased less, 1.8-fold, in the dnaA3455 cells indicating that DnaAs decreased repressor activity results in a decreased change in gene expression in the presence of LexA. This is not the case in the ΔlexAΔsulA dnaA3455 cells after UV treatment (Figure 6A), indicating that LexA is required for UV-dependent SOS induction but not DnaA. These results are consistent with the need of both DnaA and LexA to maintain a high level of repression in the absence of UV treatment.

The Simultaneous Absence of Both LexA- and DnaA-Dependent Repression on Transcription Results in Elongated Cells With Incomplete DNA Replication

To clarify the role of DnaA-dependent repression on SOS-response genes and its link with DNA replication, we compared nucleoids and cell size in the dnaA3455, ΔlexAΔsulA and ΔlexAΔsulA dnaA3455 cells with that of the wild-type cells. Flow cytometry analysis showed that the wild-type cells had four or eight fully replicated chromosomes, after rifampicin and cephalin treatment (Figure 6B). The DNA histogram of dnaA3455 showed well-separated two-, three- and four-chromosome peaks (Figure 6B), indicating that the mutant cells contain fully replicated chromosomes although initiation of replication is asynchronous (Gon et al., 2006). However, the
FIGURE 6 | Continued
that from non-treated cells as indicated by the open bars. The values shown at top of the bars are the average of three individual experiments, and the standard errors are shown. ***Stands for P-value ≤ 0.01, **for 0.01 < P-value ≤ 0.05, and ns represents P-value > 0.05. (B) Exponentially growing cells were treated for 4–5 generations with rifampicin and cephalaxin to inhibit both initiation of replication and cell division but allowing ongoing replication finish. Then cells were analyzed by flow cytometry after staining with Hoechst 33258 for 30 min. The X-axis indicates chromosome equivalents per cell, the Y-axis represents the number of cells measured. Each measurement includes 10000 cells. The doubling time and genotype of the cells are shown. (C) Exponentially growing cells were harvested and fixed in 70% ethanol. Cells after staining in Hoechst 33258 for 30 min were visualized by Zeiss LSM710 confocal microscope as described in section “Materials and Methods.” The blue structures indicate nucleoids and the red scale bar represents 2 µm.

**lexA**Δ**sulA** cell culture showed that a portion of the cells contained a DNA amount between four- and eight-chromosome after rifampicin and cephalaxin treatment, indicating that a portion of **ΔlexA ΔsulA** cells has incomplete replication, probably due to overexpression of DNA repair proteins slowing down the replication forks (Figure 6B). The phenotype of incomplete replication was worsened in the **ΔlexA ΔsulA dnaA**A345S cells. Some cells had only one-chromosome while other cells had more DNA than eight-chromosome equivalents (Figure 6B). These results underline the need for the wild-type DnaA to control both initiation of DNA replication and gene expression when the SOS response is activated. These results suggest that the **ΔlexA ΔsulA dnaA**A345S cells have more serious DNA damage, even in the absence of UV irradiation, since severe incomplete replication can be caused from lack of replication elongation or/and partial degradation of the DNA (Skarstad and Boye, 1993; Morigen et al., 2003).

Exponentially growing cells were fixed in 70% ethanol and visualized under Zeiss LSM710 Confocal microscope. As shown in Figures 6B,C, both the wild-type and **ΔlexA ΔsulA** cells were 2.4 ~ 3.0 µm in length with a similar doubling time of 26 ~ 27 min, containing mostly well-compacted one or two nucleoids. The **dnaA**A345S cells were about 4.5 µm in length with a doubling time of 34 min and more nucleoids per cell. The **ΔlexA ΔsulA dnaA**A345S cells were further elongated (about 5.5 µm) with a slower growth, and their multi-nucleoids were not well-compacted (Figures 6B,C and Supplementary Figure S2). These results together suggest that the simultaneous absence of both LexA- and DnaA-dependent repression of gene transcription results in production of elongated cells with incomplete replication of DNA, aberrant nucleoids and slower growth. It is likely that DnaA-dependent repression of gene transcription during the SOS response is required to prevent over-expression of the SOS regulon genes to maintain genome integrity.

**Transcriptions of the recN and dinJ Genes Are Also Repressed by Both DnaA and LexA**

To test whether DnaA is also involved in regulation of other SOS regulon genes (Finch et al., 1985; Ruangprasert et al., 2014),
we searched for DnaA-box and LexA-box in the dinJ and recN genes and found that both genes had LexA-boxes and a DnaA-box around the transcription start sites as shown in Supplementary Figure S3. We then inserted the lacZ reporter gene downstream of the chromosomal recN or dinJ genes, resulting in recN-lacZ or dinJ-lacZ derivatives of the MC4100 strain. The recN-lacZ or dinJ-lacZ allele was transferred to the dnaA_{A345S}, ΔlexAΔsulA, ΔlexAΔsulA dnaA_{A345S} and lexA3 cells. As shown in Figures 7A,B, transcriptions of both the recN and dinJ genes were about 2–3-fold higher in the dnaA_{A345S}, ΔlexAΔsulA and lexA3 dnaA_{A345S} cells, respectively, indicating that transcription of recN or dinJ is repressed by both DnaA and LexA, and that the DnaA- or LexA-dependent repression of gene expression is independent. As expected, the increase in transcription of recN or dinJ was strengthened in the ΔlexAΔsulA dnaA_{A345S} cells and it was significant relative to that in the ΔsulAΔlexA cells (Figure 7 insets), suggesting that repression from DnaA and LexA is additive. The observation of an overlapping LexA-box with a DnaA-box in the recN or dinJ promoter (Fernandez De Henestrosa et al., 2000). We conclude that DnaA is, indeed, involved in control of other genes of the SOS regulon.

**DISCUSSION**

The UvrB protein is a very important protein in the response to DNA damage in prokaryotic cells, performing NER with UvrA and UvrC (Truglio et al., 2006). UvrB paralogs have been found in all organisms (Sancar and Reardon, 2004) and the NER repair system plays a central role in maintaining genome integrity. Defects in NER cause several lines of diseases in humans including skin cancers (Lehmann, 2003). However, the control mechanism of uvrB gene expression remains elusive. The results presented here show that the DNA replication initiator, the DnaA protein, and the SOS regulator LexA, regulate the expression of the uvrB gene by interacting with the uvrB promoter region. The regulation mode by both DnaA and LexA applies to the expression control of several SOS genes, and may be conserved in Gram-negative bacteria. However, this hypothesis requires further experiments to be confirmed.

**DnaA and LexA Regulate Transcription of the uvrB Gene By Binding to Their Specific Sites**

We have shown that transcription from the uvrB promoters is repressed by the presence of both the wild-type LexA and DnaA proteins (Figure 1A). The full activity of the uvrB promoter cluster requires both uvrBp1-2 and uvrBp3 promoters and is repressed by both DnaA and LexA in an additive manner (Figures 4B–D). The DNase I footprinting experiments show that both proteins bind to the promoter region and that LexA-box1 in uvrBp1-2 is the strongest LexA-box from which LexA is not easily displaced by increasing amounts of ATP-DnaA, unlike what is observed at the other LexA-box in this region (Figure 3). LexA-box1 has a typical consensus sequence containing both CTG and CAG trimers at the two ends (Walker, 1984; Fernandez De Henestrosa et al., 2000; Wade et al., 2005) while LexA-box2, 3, and 4 do not. Mutations of LexA-box1 and DnaA-box6 in fact show a strong effect on promoter activity in vivo (Figure 5). The results indicate that binding of LexA or DnaA to LexA-boxes or DnaA-boxes in the uvrB promoter region results in a direct control of promoter activity.
The *uvrBp3* promoter is interesting because it completely overlaps with the DARS1 sequence. While the DARS1 sequence is not essential, in its absence initiation of DNA replication occurs at an increased cell mass (Fujimitsu et al., 2009). Binding of LexA to DARS1 and RNA polymerase to the *uvrBp3* promoter could both compete with DnaA binding and thus interfere with the regeneration of ATP-DnaA both in the absence (intact LexA) and the presence (RNAP binding) of the SOS response. This can explain the increased loss of repression by the DnaA-box6 mutation in the ΔlexAΔsulA strain (Figure 5B).

**DnaA and LexA May Coordinate DNA Replication With DNA Repair**

LexA-dependent regulation of DARS1 activity is only one of several processes resulting in an increased ATP-DnaA to ADP-DnaA ratio following DNA damage. Upon prolonged stress, fork stalling and blockage of DNA replication, ATP-DnaA accumulates in the cell (Kurokawa et al., 1999). Hydrolysis of the ATP bound to DnaA is mediated by the RIDA process, which requires ongoing DNA replication (Katayama et al., 1998). When the DNA replication forks stall in the presence of DNA damage ATP-DnaA can thus accumulate in the cell and bind to the other sites on the genome. The longer DNA replication has been stalled, the more ATP-DnaA has accumulated in the cell. This would ensure that the expression of DNA repair proteins by the SOS response is limited when the DNA replication forks have stalled and there is less DNA per cell. Furthermore, expression of the *dnaA* gene is induced by DNA damage in a RecA and LexA-dependent manner despite the absence of a LexA-box at the *dnaA* promoter region (Quinones et al., 1991). In these conditions SeqA has been shown to play a key role in cell survival, possibly by limiting over-initiation of DNA replication by increased amounts of ATP-DnaA in the presence of stalled replication forks (Sutera and Lovett, 2006). The increase in ATP-DnaA by decreased RIDA, increased gene expression and increased DARS1 activity occur at the same time as loss of repression by LexA. This may appear to be inconsistent, since they both repress gene expression at several shared targets. However, it is possible that upon DNA damage LexA cleavage results in a
rapid loss of repression while the increase in ATP-DnaA occurs with a time delay, due in part to protein synthesis, resulting in a pulse of transcription activity, which, however, is proportional to the number of replication forks, due the amount of ATP-DnaA-dependent repression observed in the absence of DNA damage. It appears to be a situation similar to the hyperinitiation stress observed during oxidative damage (Charbon et al., 2014), but as a sensible response to the problem.

DnaA-Dependent Transcription Repression During the SOS Response Is Required for Maintaining Genome Integrity: A Model for Control of the SOS Regulon by LexA and DnaA

As a summary, we propose a model for the control of the SOS regulon by DnaA and LexA (Figure 8). Both DnaA and LexA repress expression of the SOS genes when the SOS response is off. When the SOS response is triggered due to DNA damage, LexA is self-cleaved (Little et al., 1980), consequently LexA-repression is removed to derepress expression of the SOS genes to repair the DNA damage (Sancar et al., 1982). During the SOS response, DnaA-dependent repression still works and largely limits the expression of the SOS genes, resulting in cells with normal nucleoids and growth rate but minor incomplete replication. The latter can be the indication of the DNA repair process since it includes controlled nicking and digestions of the DNA. The simultaneous absence of DnaA- and LexA-repression leads to cell elongation with serious incomplete replication, uncompacted nucleoids and slow growth (Figures 6B,C), possibly as a result of a further increase in expression of SOS dependent genes (Figures 1A, 7A,B). Obviously, the high level expression of the SOS genes is harmful, causing physiological problems in different cell processes. Among these problems, incomplete DNA replication can be caused by either lack of replication elongation or partial degradation of the DNA (Skarstad and Boye, 1993; Morigen et al., 2003) as a result of DNA damage. It is reasonable to consider that an excess of DNA repair proteins might "repair" DNA regions where the repairs are unwanted, resulting in DNA damage. Indeed, for example overproduction of DinB has been shown to be lethal (Margara et al., 2016). It is likely that DnaA-dependent repression of the transcription of SOS genes during the SOS response is required to prevent over-expression of the SOS genes to maintain genome integrity.

Interestingly, 13 DnaA-boxes are found in potential LexA-boxes on the E. coli chromosomes (Fernandez De Henestrosa et al., 2000). In further analysis, the overlapping LexA-box with a DnaA-box in the uvrB or recN promoter was found in several Gram-negative bacteria including Salmonella typhimurium, Serratia marcescens, Citrobacter rodentium, Klebsiella pneumoniae and Yersinia enterocolitica (Supplementary Table S1). These results suggest that DnaA is likely involved in regulation of the SOS regulon, reducing expression of the SOS genes during the SOS response in a manner that is coupled with DNA replication. The control mode may be conserved within Gram-negative bacteria.

AUTHOR CONTRIBUTIONS

M, W, and BS conceived and designed the experiments. W, G, EB, SW, and HS performed the experiments. M, W, BS, LF, and YS analyzed the data. M and BS provided reagents. M, W, and BS wrote the manuscript. All authors read the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.01212/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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