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Negative regulation of DNA repair gene (*uvrA*) expression by ArcA/ArcB two-component system in *Escherichia coli*

Hiroshi Ogasawara [a], Jun Teramoto [a], Satoshi Yamamoto [a], Kiyo Hirao [a], Kaneyoshi Yamamoto [a], Akira Ishihama [b], Ryutaro Utsumi [a,*]

[a] *Department of Bioscience and Biotechnology, Graduate School of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan*
[b] *Division of Molecular Biology, Nippon Institute for Biological Science, Ome, Tokyo 190-0024, Japan*

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Abstract

The ArcA/ArcB two-component signal transduction system of *Escherichia coli* regulates gene expression in response to the redox conditions of growth. In this study, *uvrA* gene expression was repressed when ArcA was induced in *E. coli*. Transcription of *uvrA* increased in *ΔarcA* and *ΔarcB* strains more than in the wild-type strain, whose trend was remarkable under the anaerobic condition. In the wild-type strain grown in the presence of DTT (10 mM), the *uvrA* gene expression was also repressed. Furthermore, the results of in vitro transcription and DNase I footprinting experiments indicated that ArcA specifically bound to the ArcA box [(A/T)GTTAATTA(A/T)] in the *uvrA* promoter and represses its transcription. These results suggest that the ArcA/ArcB two-component system works to negatively regulate *uvrA* gene expression.

Keywords: *uvrA*; *ssb*; ArcA/ArcB; Two-component system; *Escherichia coli*

1. Introduction

The ArcA/ArcB two-component system (TCS) of *Escherichia coli* regulates the expression of about 30 operons that respond to redox conditions [1–10]. In addition to these redox-responsive genes, at least three targets that are not involved in the respiratory metabolism have been found to be under the control of ArcA/ArcB. These targets are the *tra* operon for plasmid conjugation, the *psi* site for *Xer*-based recombination, and the *oriC* site for chromosome replication [11–13]. Recently, Liu and De Wulf [14] identified, using a DNA microarray and the AlignACE program, as many as 55 new Arc-regulated operons under anaerobic growth conditions, indicating that the Arc-controlled pathway recruits a total of 100–150 operons for adaptation to the availability of external oxygen [11]. The *uvrA* gene encodes the nucleotide excision-repair protein UvrA and is induced as part of the SOS response [15]. Gifford et al. [16] demonstrated that when cells were shifted from anaerobic to aerobic growth, the transcript levels of the *uvrA* increased. A systematic DNA microarray analysis of 36 TCS mutants by Oshima et al. [17] suggested that the gene (*uvrA*) involved in DNA repair is also regulated by ArcA/ArcB TCS. These results suggest that the ArcA/ArcB two-component system plays a role in controlling the *uvrA* gene in response to the respiratory growth conditions independent of the SOS system.

* Corresponding author. Tel.: +81 742 43 7306; fax: +81 742 43 8976.
E-mail address: utsumi@nara.kindai.ac.jp (R. Utsumi).

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Thus, we tried to clarify the detailed molecular mechanism of *uvrA* gene expression by ArcA/ArcB TCS.

2. Materials and methods

2.1. Bacterial strains and growth conditions

E. coli BW25113 (*lacI*^{q}, *rrnB*$_{T14}$, Δ*lacZ*$_{WJ16}$, *hsdR514*, Δ*araBAD*$_{AH33}$, Δ*rhaBAD*$_{LD78}$) [18,19], BW26422 (BW25113, Δ(*arcB*) 41), and BW27422 (BW25113, Δ(*arcA*) 43) [17] were cultured in a Luria–Bertani (LB) medium (pH 7.5) containing 1% Bacto Tryptone (Difco), 0.5% Bacto Yeast Extract (Difco), and 1% NaCl for RNA preparation. MYuvrA (MC4100, Φ*uvrA*::*lacZ*) was constructed as described [20]. After pRSuvrA was recombined with phage λRS45, MC4100 was lysogenized to isolate MYuvrA on an LB agar plate containing kanamycin (25 μg/ml). MYuvrAΔarcA and MYuvrAΔarcB were constructed by P1 transduction from donor strains BW27422 (BW25113, *arcA43*::Cam^r) and BW26422 (BW25113, *arcB41*::Cam^r), respectively, to the recipient MYuvrA.

2.2. Construction of plasmids

A DNA fragment (530 bp) containing the *uvrA* promoter region was prepared by polymerase chain reaction (PCR) using *E. coli* BW25113 genome DNA as a template and a pair of primers, uvrA-EcoRI-F (5′-CCTTTACGCAGGAATTCGCTCGCCA-3′) and uvrA-BamHI-R (5′-TTGTCGCGGGGGATCCCGAGGTTGA-3′). After digestion with *Eco*RI and *Bam*HI, the PCR-amplified fragment was inserted at the corresponding site of pRS551 [20] to generate the plasmid pRSuvrA. A DNA fragment (827 bp) containing the *arcA* coding region was prepared by PCR using *E. coli* BW25113 genome DNA as a template along with a pair of primers, arcA-BAD-EcoRI-F (5′-GACTTTTGGAATTCCTGTTTCGATT-3′) and arcA-BAD-XbaI-R (5′-GAGTTACGTATCTAGAAATAAGGTT-3′). After digestion with *Eco*RI and *Xba*I, the PCR-amplified fragment was inserted at the corresponding site of pBAD18 [21] to generate the plasmid pBADarcA. A *Bam*HI–*Not*I fragment (698 bp) of ArcA, was cloned into the corresponding sites of pET-21a(+) (Novagen) to construct the plasmid pKH50-3 [22].

2.3. Preparation of labeled probes for S1 nuclease, gelshift and DNase I footprinting assays

Probe A was generated by PCR amplification of the *uvrA* promoter region with the primers, uvrA-S1F (5′-GTCCTGACCCAGATTACCAA-3′) and ^{32}P-labeled uvrA-S1R (5′-CGAGGTTGATGTTTTTGAGA-3′), and *E. coli* BW25113 genome DNA (100 ng) as the template for the Ex Taq DNA polymerase. The PCR product with ^{32}P at its terminus was recovered from the polyacrylamide gel after electrophoresis and then used for S1 nuclease assay (transcription of *uvrA*) and DNase I footprinting assay. The uvrA-S1R and labeled uvrA-S1F primers were used to prepare probe B for an S1 nuclease assay (transcription of *ssb*) and DNase I footprinting assay. The mfd-S1F (5′-GCATTCAGCGATCTGTTGT-3′) and ^{32}P labeled mfd-S1R (5′-GGCGTGACGTTCGGCAATTT-3′) primers were used to prepare probe C for S1 nuclease assay (transcription of *mfd*). The cydA-S1F (5′-GGTCAACCGTGCTGTTTTTGCTTCGT-3′) and ^{32}P labeled cydA-S1R (5′-GCTCATCGCATGAAGACTCCGAGAGT-3′) primers were used to prepare probe D for S1 nuclease assay (transcription of *cydA*). The labeled primers were prepared with 10 μCi of [γ-^{32}P]ATP (5000 Ci/mmol) by T4 polynucleotide kinase (Toyobo) as described previously [23].

2.4. RNA isolation and S1 nuclease assay

To prepare total RNA for the S1 nuclease assay, overnight cultures were diluted 100-fold in the LB medium and grown under both aerobic and anaerobic conditions. In the aerobic growth condition, cells were grown in the LB medium at 37 °C by reciprocal shaking (160 rpm, stroke: 3 cm) in a 300-ml round-bottom flask (Iwaki Co., Japan) containing 50 ml of medium. In the anaerobic condition, cells were grown in the LB medium at 37 °C under the cap-sealed condition in a 300-ml narrow-mouth flask (Iwaki Co., Japan) containing 300 ml of medium. Cells were harvested during the logarithmic growth phase (OD_{600} of 0.4) to prepare total RNA. Subsequent purification steps were carried out as described previously [23]. Probe A, B, C, or D (10,000 cpm, 2 fmol) was incubated with 100 μg of total RNA in hybridization buffer (80% formamide, 0.4 M NaCl, 20 mM Hepes [pH 6.4]) at 75 °C for 10 min, followed by further incubation at 37 °C overnight and then digestion with 50 units of S1 nuclease. The undigested DNA was precipitated by ethanol, dissolved in formamide dye solution (95% formamide, 0.05% bromophenol blue and 0.05% xylenecyanol), and analyzed by electrophoresis on a 6% polyacrylamide gel containing 8 M urea.

2.5. Purification of ArcA

His-tagged ArcA for DNase I footprinting and in vitro transcription assays was purified from BL21 (DE3) containing pKH50-3 as described previously [22,24].

2.6. Gel shift assay

Probe A, B, or D (10,000 cpm, 2 fmol) was incubated at 37 °C for 10 min with ArcA in 12.5 ml of 50 mM

Tris–HCl (pH 7.8), 50 mM NaCl, 3 mM Mg acetate, 0.1 mM EDTA, and 0.1 mM dithiothreitol. After addition of the DNA dye solution (40% glycerol, 0.025% bromophenol blue and 0.025% xylenecyanol), the mixture was directly subjected to 6% polyacrylamide gel electrophoresis.

2.7. DNase I footprinting assay

Either 40,000 cpm of probe A or B (8 fmol) was incubated at 37 °C for 10 min with ArcA in 25 μl of 50 mM Tris–HCl (pH 7.8), 50 mM NaCl, 3 mM magnesium acetate, 5 mM $CaCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 25 mg/ml BSA. After incubation for 10 min, DNA digestion was initiated by the addition of 5 ng of DNase I (Takara). After digestion for 30 s at 25 °C, the reaction was terminated by the addition of 45 μl of a DNase I stop solution (20 mM EDTA, 200 mM NaCl, 1% sodium dodecyl sulfate, 250 μg/ml yeast tRNA). Digested products were precipitated by ethanol, dissolved in formamide dye solution, and analyzed by electrophoresis on 6% polyacrylamide gel containing 8 M urea.

2.8. In vitro transcription assay

A 530-bp *Eco*RI–*Bam*HI fragment from pRSuvrA, including the *uvrA* promoter (−128 to +402), or a 205-bp *Eco*RI fragment from pKB252 [25], including the *lac*UV5 promoter (−65 to +140), was used as the template DNA. Single-round transcription by the reconstituted holoenzymes was carried out as described previously [26]. Briefly, 0.1 pmol of template was incubated with 0, 2.5, 5, 10, and 20 pmol ArcA for 10 min at 37 °C in a total volume of 33 μl. Into this reaction mixture, 0.5 pmol RNA polymerase was added and incubated for 20 min at 37 °C to form an open complex. Then a substrate/heparin mixture containing [γ-^{32}P]UTP was added and further incubated for 10 min at 37 °C. The transcripts were subjected to 6% polyacrylamide gel containing 8 M urea for electrophoresis.

3. Results and discussion

3.1. Negative regulation of uvrA gene expression by ArcA/ArcB two-component system

To clarify the effect of ArcA on *uvrA* gene expression, *E. coli* MYuvrA containing pBADarcA was cultured in the presence or absence of arabinose, and *uvrA* gene expression was measured as β-galactosidase activity. As a result, *uvrA* expression was remarkably repressed by induction of ArcA with arabinose (Fig. 1A), but in the *arcA* and *arcB*-defective strains, it increased more than in the wild-type strain (Fig. 1B).

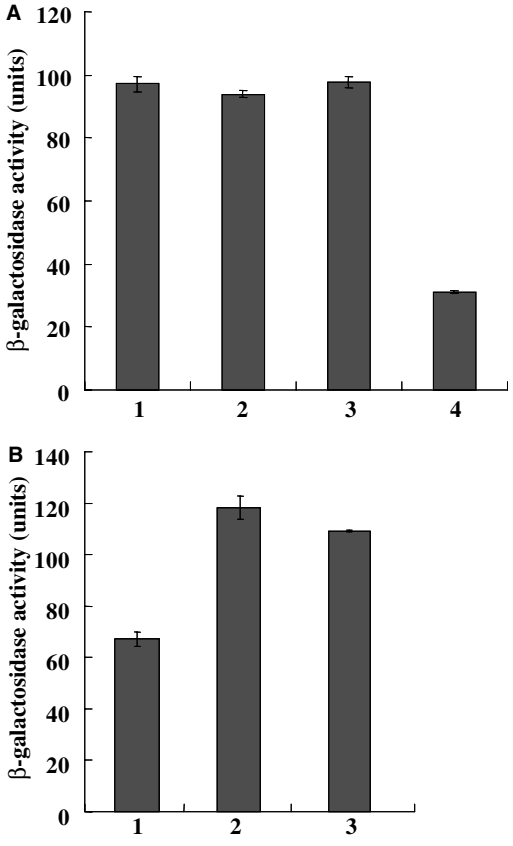

Fig. 1. Involvement of ArcA/ArcB two-component system in *uvrA* gene expression. (A) After *E. coli* MYuvrA (pBAD18) (lanes 1 and 2) and MYuvrA (pBADuvrA) (lanes 3 and 4) were grown in LB medium to OD_{600} of 0.4, they were incubated in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of arabinose (0.2%) for 20 min at 37 °C to determine β-galactosidase activity [30]. (B) When *E. coli* MyuvrA (lane 1), MYuvrAΔarcA (lane 2) and MYuvrAΔarcB (lane 3) were grown in LB medium to an OD_{600} of 0.6, β-galactosidase activity was assayed. Data represent the means of duplicate values with standard deviations.

To confirm that *uvrA* gene expression was regulated negatively by the ArcA/ArcB two-component system, we performed S1 nuclease assays of RNAs prepared from BW27422 (BW25113 Δ*arcA*) [17,19], BW26422 (BW25113 Δ*arcB*) [17,19], and the wild-type strain (BW25113) [18] under both aerobic and anaerobic growth conditions.

Consequently, transcription of *uvrA* increased in BW27422 and BW26422 more than in BW25113, whose trend was remarkable under the anaerobic condition (Fig. 2A, B). On the other hand, *cydA gene expression* decreased in BW27422 and BW26422 more than in BW25113 (Fig. 2C, D) as described previously [27].

When ArcA was overexpressed in BW25113 containing pBADarcA, the transcription of *mfd* (control) was not affected (Fig. 3C, lanes 3 and 4), but the transcription of *uvrA* was markedly repressed compared to the case in the absence of arabinose (Fig. 3A, lanes 3 and 4), while the level of *uvrA* transcript did not change in

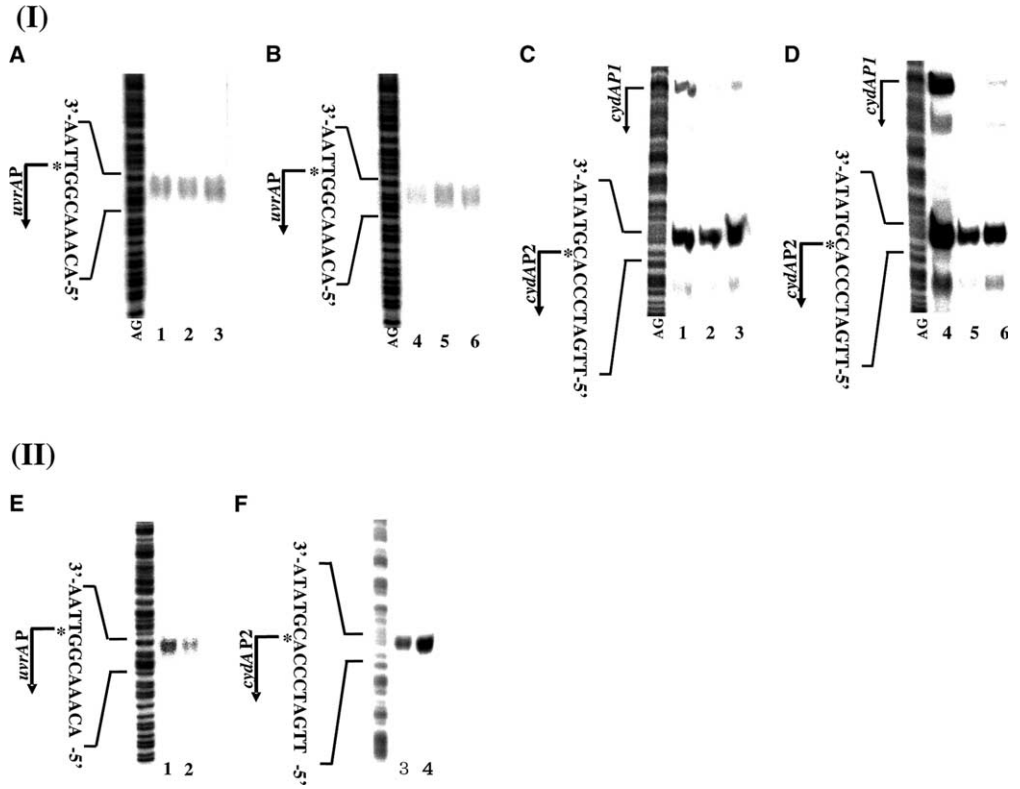

Fig. 2. Effect of respiratory condition on *uvrA* and *cydA* gene expressions. In Panel I, BW25113 (wild type) (A, B, C, D, lanes 1 and 4), BW27422 (Δ*arcA*) (A, B, C, D, lanes 2 and 5), and BW26422 (Δ*arcB*) (A, B, C, D, lanes 3 and 6) were grown to early-log phase (OD_{600} of 0.4) under an aerobic (lanes 1, 2, and 3) or anaerobic (lanes 4, 5, and 6) condition. After that, S1 nuclease assays for *uvrA* (A, B) and *cydA* (C, D) transcripts were performed using the probes A and D, respectively (see Materials and Methods). In Panel II, BW25113 was grown aerobically in an LB medium containing 0.1 M MOPS (pH 7.4) and 20 mM D-xylose to early-log phase (OD_{600} of 0.4) in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 10 mM DTT. After that, S1 nuclease assays for *uvrA* (lanes 1 and 2) and *cydA* (lanes 3 and 4) transcripts were performed using probes A and D, respectively. Lane AG represents the Maxam-Gilbert sequence ladder. The transcription start site is marked with an asterisk.

the presence or absence of arabinose in BW25113 containing vector pBAD18 (Fig. 3A, lanes 1 and 2).

3.2. uvrA *gene expression under reducing condition*

To show that *uvrA* gene expression is regulated under reducing conditions (e.g. in the presence of DTT), we investigated the effects of DTT on *uvrA* gene expression (Fig. 2E, F). As the result, when the wild-type *E. coli* was incubated in the presence of 10 mM DTT aerobically, *uvrA* gene expression was repressed (Fig. 2E, lane 2), but *cydA* gene expression increased as previously reported [28] (Fig. 2F, lane 2).

3.3. *Negative regulation of* ssb *transcription by ArcA*

The *ssb* gene encoding single-strand DNA-binding protein is located next to *uvrA* but transcribed in the opposite direction (see Fig. 6). Both *uvrA* and *ssb* genes are organized in the SOS regulon and regulated by the LexA repressor [15,29]. We then examined the possible influence of ArcA on *ssb* transcription. From S1-mapping, we detected three transcripts, indicating the presence of three promoters (P1, P2 and P3 in this order from downstream) (Fig. 3B). When ArcA was overexpressed in BW25113 containing pBADarcA in the presence of 0.2% arabinose, the transcription from ssb_{P3} decreased to an undetectable level, and that from ssb_{P1} decreased by 3-fold (Fig. 3B, lane 4) compared to that of BW25113 containing pBAD18 (Fig. 3B, lane 2).

3.4. *In vitro transcription of* uvrA *and* ssb

For detailed analysis of the repression mechanism of *uvrA* and *ssb* transcription by ArcA, in vitro transcription using RNA polymerase holoenzyme $E\sigma^{70}$ and truncated DNA templates containing either the *uvrA* or *ssb* promoter region were carried out in the presence or absence of ArcA. The addition of ArcA significantly caused repression of transcription from the *uvrA* promoters in a dose-dependent manner but did not affect the expression from the *lac* UV5 promoter (Fig. 4). In good agreement with the in vivo observations (see Fig. 3), transcription of *ssb* was also repressed by ArcA (Fig. 4). The addition of ArcA caused marked repression of transcription from ssb_{P3} and weaker repression of transcription from ssb_{P1} and ssb_{P2}.

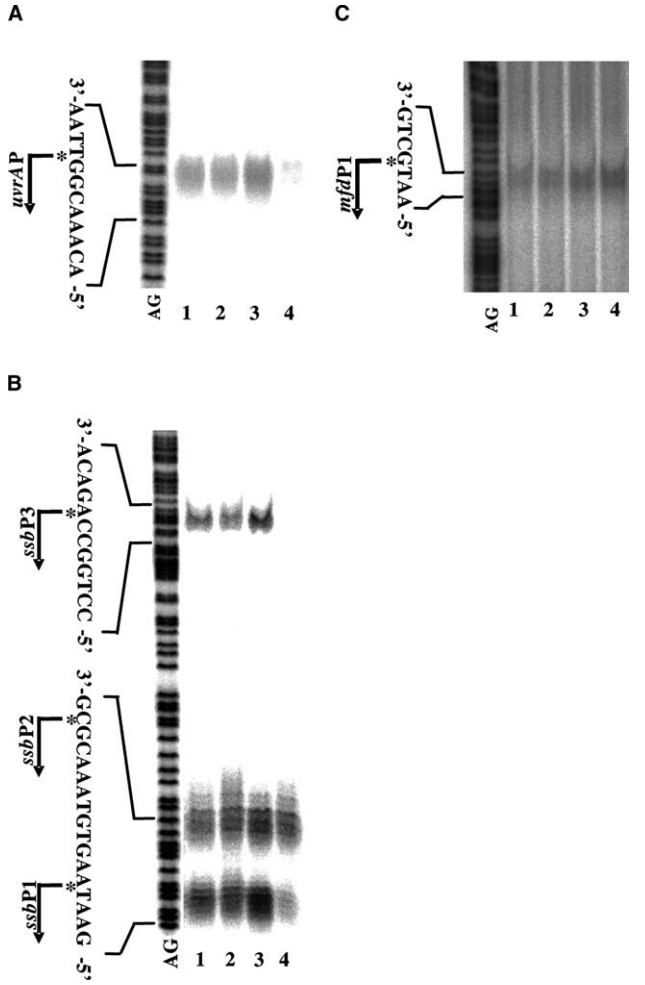

Fig. 3. Effect of overexpressed ArcA on *uvrA* and *ssb* transcriptions. BW25113 containing pBAD18 (A, B, C, lanes 1 and 2) and BW25113 containing pBADarcA (A, B, C, lanes 3 and 4) were grown to early-log phase (OD_{600} of 0.4) in the absence (A, B, C, lanes 1 and 3) or presence (A, B, C lanes 2 and 4) of 0.2% arabinose. S1 nuclease assays for *uvrA* (A), *ssb* (B), and *mfd* (C) transcripts were performed as described in the text. Lane AG represents the Maxam-Gilbert sequence ladder. The transcription start site is marked with an asterisk.

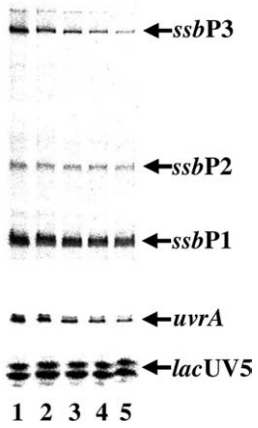

Fig. 4. In vitro transcription assay. Single-round transcription *in vitro* was performed with a 0.1-pmol *uvrA-ssb* DNA template (−128 to +402 of *uvrA* promoter region) and a *lac* UV5 DNA template. The amounts of ArcA were as follows: lane 1, 0 pmol; lane 2, 2.5 pmol; lane 3, 5 pmol; lane 4, 10 pmol; lane 5, 20 pmol. Electrophoresis was performed with a 6% polyacrylamide sequencing gel. Bold arrows indicate the *uvrA*, ssb_{P1}, ssb_{P2}, ssb_{P3} and *lac* UV5 transcripts.

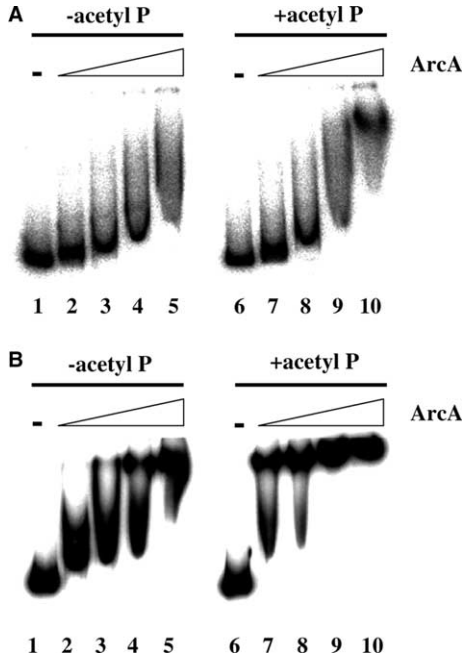

Fig. 5. Gel shift assays. (A) Probe A for *uvrA* promoter was incubated at 37 °C for 10 min with ArcA (lanes 1–5) or ArcA phosphorylated by acetylphosphate at 37 °C (lanes 6–10). The amounts of ArcA were as follows: lanes 1 and 6, 0 pmol; lanes 2 and 7, 1.25 pmol; lanes 3 and 8, 2.5 pmol; lanes 4 and 9, 5 pmol; lanes 5 and 10, 10 pmol. (B) Probe D for *cydA* promoter was incubated at 37 °C for 10 min with ArcA (lanes 1–5) or ArcA phosphorylated by acetylphosphate at 37 °C (lanes 6–10). The amounts of ArcA were as follows: lanes 1 and 6, 0 pmol; lanes 2 and 7, 1.25 pmol; lanes 3 and 8, 2.5 pmol; lanes 4 and 9, 5 pmol; lanes 5 and 10, 10 pmol.

3.5. Identification of ArcA-binding sites

A gelshift experiment resulted in enhancement of the ArcA-promoter complex for *uvrA*/*ssb* and *cydA* promoter fragments in the presence of acetylphosphate (Fig. 5), while ArcA was able to bind to *uvrA*/*ssb* and *cydA* promoters in the absence of acetylphosphate.

To define the mechanism underlying the repression of *uvrA* and *ssb* transcription by ArcA, we tried to identify the specific ArcA-binding site on these promoters by using DNase I footprinting assay. ArcA was found to bind to the *uvrA* promoter between −19 and +16 (A1: ArcA site-1; −56 and −90 with respect to *ssb* P3 promoter) (Fig. 6A). These ArcA-binding regions include the ArcA-binding motif 5′-A/TGTTA-ATTAA/T-3′ [7].

Detailed footprinting analysis of ArcA on the *ssb* promoters indicated additional ArcA-binding sites at −80 and −95 (A2: ArcA site-2; between −61 and −76 with respect to the *uvrA* promoter), between −41 and

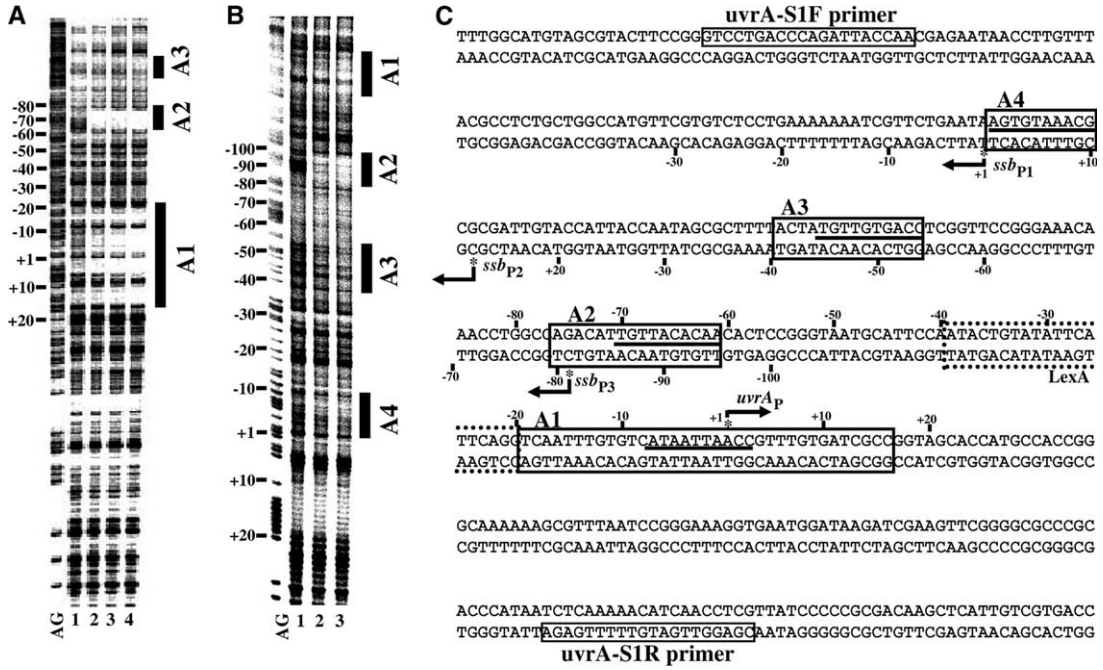

Fig. 6. ArcA-binding sites on the *uvrA* and *ssb* promoter region. (A) DNase I footprinting assay of the ArcA to the *uvrA-ssb* promoter region. Probe A (panel A) or probe B (panel B) was incubated with various amounts of the purified ArcA (lane 1, 0 pmol; lane 2, 20 pmol; lane 3, 40 pmol; lane 4, 80 pmol) and subjected to DNase I footprinting assays. Lane AG represents the Maxam-Gilbert sequence ladder. Black boxes indicate the ArcA-binding region. (C) Transcription start sites are marked with asterisks and arrows. The nucleotide number represents the distance from the transcription initiation sites of the *uvrA*, *ssb* P1 promoters. The LexA-binding region is boxed by the dotted line. The ArcA-binding region is also boxed; the bold line indicates the ArcA-binding consensus region (5′-A/TGTTAATTAA/T-3′).

−54 (A3: ArcA site-3; −102 and −115 with respect to the *uvrA* promoter), and between −1 and −10 (A4: ArcA site-4; −146 and −155 with respect to the *uvrA* promoter) upstream from the ssb_{P1} transcriptional start position (Fig. 6B). All of these regions include the ArcA-binding motif 5′-A/TGTTAATTAA/T-3′ [7], showing that the divergently transcribed *ssb* and *uvrA* genes are controlled by these ArcA-binding sites.

Acknowledgments

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