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Aggregation of SeqA protein requires positively charged amino acids in the hinge region

Sukhyun Kang [a,1,2], Joo Seok Han [a,2], Sung Ho Kim [b,2], Jong Hoon Park [a,2], Deog Su Hwang [a,b,*]

[a] *Institute of Molecular Biology and Genetics, Seoul National University, Seoul 151-742, Republic of Korea*
[b] *Department of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea*

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Abstract

SeqA proteins of *Escherichia coli* bound to the hemimethylated GATC sequences (hemi-sites) interact with each other and eventually form an aggregate. SeqA foci, which are suggested to be formed by aggregation, play important roles in the regulation of chromosome replication and segregation. We found that aggregation of SeqA proteins was preceded by cooperative interactions between these proteins bound to hemi-sites. Positively charged amino acids in the hinge region, which connects the N-terminal and C-terminal domain of SeqA, were critical for SeqA aggregation on hemimethylated DNA. Although the substitution of positively charged amino acids with negatively charged or neutral amino acids maintained the binding and cooperative interaction of mutant proteins, these proteins were defective in aggregation and foci formation *in vitro* and *in vivo*, respectively. Our results suggest that *in vivo* SeqA foci were formed by aggregation following cooperative interactions between SeqA proteins bound to hemi-sites.

Keywords: SeqA; Hemimethylated DNA; Aggregation; DNA–protein interaction

Replication of the chromosomal DNA initiates only once at a specific region known as the origin of chromosomal replication (*oriC*) and elongates bidirectionally [1,2]. The chromosome of *Escherichia coli* is methylated at adenine residues in GATC sequences by Dam methylase. Following the replication fork progression and the nascent strand synthesis, the daughter DNA becomes hemimethylated [3]. SeqA protein binds to the hemimethylated GATC sequences (hemi-sites) and performs various roles to control the cell cycle progression. Immediately after the initiation of replication, SeqA binds to the replicated *oriC* and sequesters it from remethylation and reinitiation of replication at the replicated *oriC* [3–7]. SeqA tracks replication forks as a multiprotein complex and contributes to the maintenance of superhelicity and decatenation of daughter chromosomes through the stimulation of topoisomerase IV [8–11].

The multiprotein complex, which was visualized as foci under *in vivo* immunostaining, appears to be formed by aggregation of SeqA on hemimethylated DNA. First, a SeqA molecule binds to two adjacent hemi-sites. The atomic force microscopy (AFM) images of the SeqA–DNA complex revealed that a SeqA molecule exhibited a dimeric configuration, in which two N-terminal multimerization domains (N-domain, amino acids 1–50) of each subunit interacted with each other to form a dimer [12]. The C-terminal domain (C-domain, amino acids 71–181) of each

Abbreviations: Hemi-site, hemimethylated GATC sequence; N-domain, N-terminal multimerization domains; C-domain, C-terminal domain.

* Corresponding author. Address: Department of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea. Fax: +82 2 873 7524.
E-mail address: dshwang@snu.ac.kr (D.S. Hwang).

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subunit, which binds to a hemi-site, was linked to the N-domain by a flexible hinge region. When another SeqA molecule binds to a pair of hemi-sites at a distance closer than 32 bp, the two SeqA molecules interact cooperatively and stabilize their binding to the DNA [13]. These two SeqA molecules acquire enhanced interaction capability and can interact with a SeqA molecule that is bound to more distant hemi-sites, up to ca. 200 bp [13]. The interaction of at least three SeqA molecules on DNA leads to further recruitment of free SeqA molecules on the protein–DNA complex, thereby forming an aggregate.

Here, we showed that aggregation of SeqA required a distinct type of interaction in which the hinge region of the protein participated. The cooperative interaction between SeqA proteins bound to hemi-sites was followed by aggregation. Mutant protein defective in aggregation failed to form foci *in vivo*.

Materials and methods

Materials. The sources were as follows: restriction enzymes and cloning enzymes, Promega; [γ-^{32}P]ATP (5000 Ci/mmol) and poly(dI-dC), Amersham Biosciences; T4 polynucleotide kinase, New England Biolabs; Pyrobest DNA polymerase and T4 DNA ligase, Takara; QIAEX II gel extraction kit, Qiagen; site-directed mutagenesis kit, Stratagene; and unmethylated or methylated synthetic oligonucleotides, Genotech. Unless otherwise stated, additional reagents were purchased from Sigma.

Proteins. The mutant forms of *seqA* were constructed by site-directed mutagenesis as described previously [14]. Wild-type SeqA and its mutants were expressed and purified from W3SQT [pBAD18-*seqA*] as described previously [14].

DNA containing hemi-sites. The sequences of the DNA fragments used are shown in Table 1. The DNA fragment containing 17 GATC sites was obtained from the pFT*oriC* plasmid by restriction with XbaI and XhoI [15]. Hemimethylated DNA was prepared from unmethylated and methylated plasmids as described previously [15].

Gel shift assays and 1,10-phenanthroline-copper ion nuclease in situ footprinting. Unless otherwise stated, the 20 μl reaction mixtures contained 10 mM Tris–HCl (pH 7.6), 50 mM KCl, 1 mM EDTA, 1 mM DTT, 1 μg of poly(dI-dC), 10% glycerol, 5 μg BSA, and ~2 fmol hemimethylated DNA with the specified amount of SeqA protein. The mixtures were incubated for 15 min at 32 °C, and the subsequent steps were performed as described previously [15]. 1,10-Phenanthroline-copper (II) (OP-Cu(II)) ion nuclease footprinting was performed after gel shift assay as described previously [4].

Topoisomerase assays. Topoisomerase IV relaxation assays were performed as described previously [11]. The reaction products were separated on 1% agarose gel at 20 V for 17−20 h, transferred to nitrocellulose membranes for Southern hybridization, and visualized by autoradiogram. Hemimethylated plasmids were generated *in vitro* by using previously described methods [11].

Construction of SeqA mutant strains and immunostaining. To construct expression cassette of *ΔseqA(K66E, R70E)*, the EcoRI/HindIII fragment containing the coding region of *seqA(K66E, R70E)* and the BamHI/EcoRI fragment containing the *seqA* promoter region were cloned together into the BamHI/HindIII site of pRS415 [16]. The resulting plasmids were transferred onto λRZ5 by *in vivo* homologous recombination. W3SQT (*seqA*::Tc^{R}) [13] was lysogenized with the obtained phage.

Immunostaining procedures were performed according to Hiraga et al. [8]. Each strain of *E. coli* was fixed with ethanol. Cells were lyzed using a lysozyme solution (2 mg/ml lysozyme in 25 mM Tris–HCl [pH 8.0], 50 mM glucose, and 10 mM EDTA) and washed three times with PBST (140 mM NaCl, 2 mM KCl, 8 mM Na_2PO_4, 1.5 mM KH_2PO_4, and 0.05% Tween 20). The cells were permeabilized with methanol/acetone and blocked with PBST containing 2% bovine serum albumin (BSA). SeqA polyclonal antibody was pretreated with the W3SQT cell extract to reduce nonspecific binding and used for immunostaining. Specimens were washed with PBST and stained with Cy3-conjugated anti-rabbit antibody. SeqA foci were analyzed by confocal microscopy (200× objective; Carl Zeiss-LSM510, Germany). Representative images of wild-type and *seqA(K66E, R70E)* staining are drawn in Fig. 4.

Results

The hinge region mutant SeqA protein is active for binding to hemi-sites and for cooperative interaction

SeqA protein exists as a homodimer and binds to a pair of hemi-sites [12]. A SeqA polypeptide is composed of two domains [12,14,17,18]. The N-domain mediates stepwise

Table 1
Nucleotide sequences of hemimethylated DNA

Hemi-sites	Figures used	DNA sequences	Plasmid	Restriction enzymes
Synthetic DNAs				
4[a]	1F	5′-gatcctgggtattaaaaagaagatctatttatttagagatctgttctattgtgatctcttattaggatcgcactgccctg-3′ 5′-gatccagggcagtgcgatcctaataagagatcacaatagaacagatctctaaataaatagatcttctttttaatacccag-3′		
Native DNAs				
2[b]	1C and D	5′-gatctgttctattgtgatctcttattaggatcgcactgccctg-3′ 5′-gatccagggcagtgcgatcctaataagagatcacaatagaaca-3′	pBMA1	BglII and BamHI
3[b]	1E	5′-gatctgttctattgtgatctcttattaggatcgcactgccctggatcccgggtaccgagctcg-3′ 5′-aattcgagctcggtacccggggatccagggcagtgcgatcctaataagagatcacaatagaaca-3′	pBMA1	BglII and EcoRI
6[b]	2A and C	5′-agcttgcatgcctgcaggtcgactctagaggatcctgggtattaaaaagaagatctatttatttagagatctgttctattgtgat ctcttattaggatcgcactgccctggatccccgggtaccgagctcg-3′ 5′-aattcgagctcggtacccggggatccagggcagtgcgatcctaataagagatcacaatagaacagatctctaaataaa tagatcttctttttaatacccaggatcctctagagtcgacctgcaggcatgca-3′	pBMA1	HindIII and EcoRI

The GATC sequences containing N^6-methyladenine are underlined. The top and bottom strands were annealed and used for DNA binding assays as described previously [15]. Native DNA fragments were prepared from fully methylated and unmethylated plasmids harboring each fragment, as described previously [15]. The hemimethylated DNA used in Fig. 2B is the XbaI/XhoI fragment of pFT*oriC* that contains the *oriC* region.

[a] The DNA sequence of the 13-mer region of *oriC* was used.

[b] These fragments were isolated from pBMA1.pBMA1 was constructed by inserting an annealed DNA fragment[a] into the BamH1 site of pUC18.

intra- and intermolecular interactions between SeqA molecules. The C-domain directly binds to hemi-sites [17,19]. The N- and C-domains are linked by the hinge region between the 51st and 73rd amino acids. This region was suggested to form coiled coils [12,17].

There are many regularly spaced positively charged amino acids between the 60th and 73th amino acids (Fig. 1A). A part of this region was suggested to be involved in SeqA–SeqA interactions [17]. To elucidate the contribution of this region to SeqA function, we substituted these positively charged amino acids and purified mutant proteins (Fig. 1B). The substitution of positively charged amino acids with negative ones rather increased the binding affinity of SeqA to a pair of hemi-sites (Fig. 1C). DNA binding of the mutant protein was also confirmed by *in situ* footprinting (Fig. 1D). Chemical nuclease protection by SeqA(K66E, R70E) was identical to that by wild-type. The binding of mutant SeqA to three or four hemi-sites was also similar to or higher than that of the wild-type protein (Fig. 1E and F). The slow mobility complex formation, particularly with three hemi-sites, indicated the ability of cooperative interactions [15]. These results suggested that the hinge region mutants were fully active for cooperative interactions.

The hinge region participates in aggregation

SeqA binds and aggregates on hemimethylated DNA when there are six or more hemi-sites, i.e., when more than three SeqA molecules are bound to DNA [15]. In this

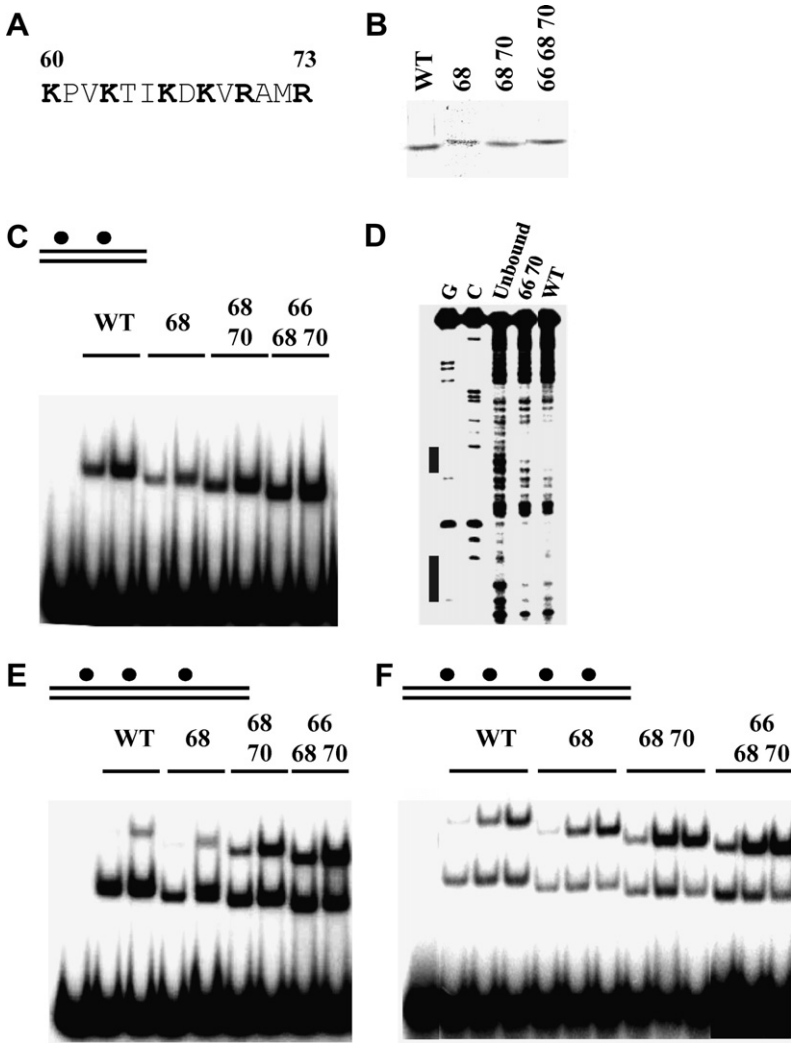

Fig. 1. Mutations in the hinge region did not decrease the binding affinity or cooperative interaction of SeqA. (A) Positively charged amino acids in the hinge region are indicated as bold capitals. (B) Purified proteins (1 μg) were separated on a 12% SDS–PAGE gel and stained with Coomassie brilliant blue R. The numbers indicate the amino acids that were substituted with Glu. DNA-binding activity of mutant proteins was analyzed by gel shift assays (C, E, and F). 10 and 40 ng of purified proteins were incubated with hemimethylated 13-mer regions containing two (C) or three hemi-sites (E). 10, 20, and 40 ng of proteins were incubated with DNA containing four hemi-sites (F). The binding sites of wild-type SeqA or SeqA(K66E, R70E) in hemimethylated DNA containing two hemi-sites were determined by *in situ* OP-Cu(II) footprinting (D). The used DNA probes are indicated by small figures on the upper portion of (C, E, and F); the closed circles represent methyladenines in GATC sequences.

study, SeqA formed aggregates at high concentrations, and these aggregates were retained near the well of the gel (Fig. 2). The aggregation property of the hinge region mutants was examined using DNA containing six hemi-sites (Fig. 2A). Similar to the wild-type protein, SeqA(K66E, R70E) mutant produced three complexes of varying mobility with an increase in concentration. However, this mutant did not form aggregates at high concentrations. Instead, there was an increase in the intensity of the third complex containing three SeqA molecules. This property was also observed with other mutants carrying charge conversion mutations in this region (data not shown). Since the complexes of SeqA(K66E, R70E) migrated faster than that of wild-type (Figs. 1 and 2A) and the mobility of the mutant polypeptide was decreased in SDS–PAGE (Fig. 1B), it is possible that the failure to form aggregates around the well resulted from the gain of mobility by increased negative charge. To rule out this possibility, we tested SeqA(K66G, R70G) in which positively charged amino acids were substituted with neutral glycine. The binding property of SeqA(K66G, R70G) was found to be similar to that of SeqA(K66E, R70E). In addition, this result excluded the possibility that aggregation defect resulted from simple charge repulsion between SeqA molecules. Comparison of binding patterns between wild-type and SeqA(K66E, R70E) to the hemimethylated *oriC* region, which contained 17 repeated hemi-sites, clearly showed that the hinge region mutants were defective in aggregation, and considerably higher concentration was required for aggregate formation (Fig. 2B).

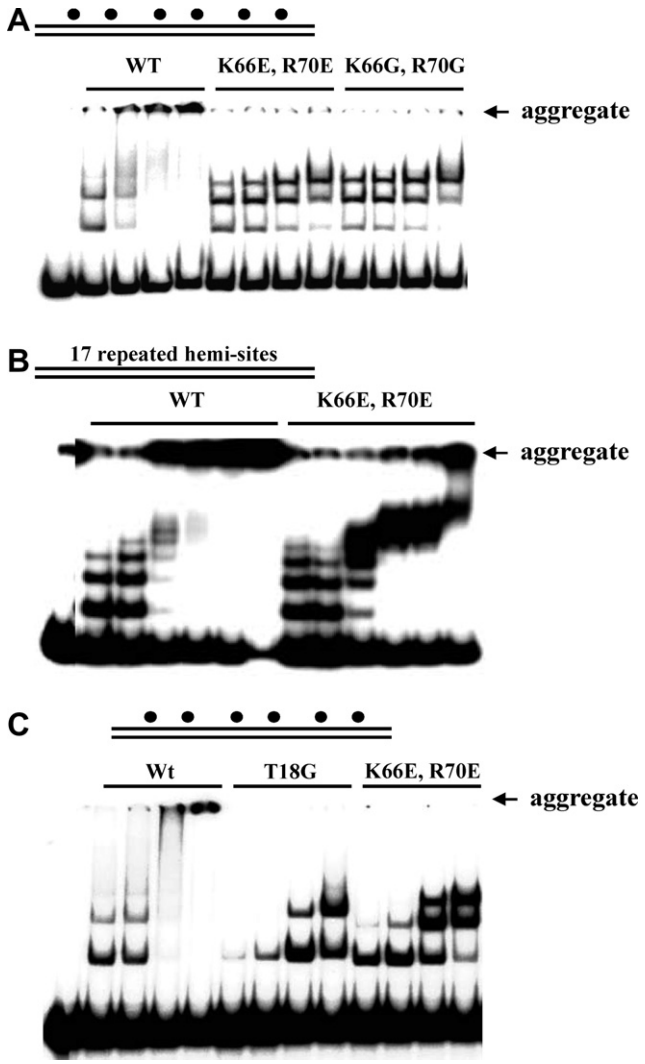

Fig. 2. Aggregation of SeqA. The hemi-site binding of wild-type or mutant SeqA proteins was measured by gel shift assay using the XbaI/XhoI fragment of pBMA1 containing six hemi-sites (A and C) or XbaI/XhoI fragment of pFT*oriC* containing 17 hemi-sites (B). The amount of indicated proteins assayed in each experiment was 10, 20, 40, and 80 ng for (A); 1, 2, 4, 8, 16, and 32 ng for (B); and 10, 20, 40, and 80 ng for (C).

We previously reported that the SeqA(T18G) mutant protein contained a defect in cooperative interaction with another SeqA protein bound to hemi-sites [12]. In comparison to the wild-type protein, the defective interaction of this mutant protein resulted in less effective formation of slower migrating complexes with relatively retarded migration. SeqA(T18G) generated weak second and third complexes with DNA containing six hemi-sites; these complexes migrated slower than those of wild-type (Fig. 2C). Unlike the wild-type protein, this mutant could not form aggregates at high concentration. The binding pattern of this mutant protein was different from that of SeqA(K66E, R70E), which was only defective in aggregation. These results implied that the aggregation of SeqA on hemimethylated DNA required cooperative interactions. This cooperative interaction preceded aggregation on hemi-sites.

SeqA(K66E, R70E) is defective in aggregation in vitro and in vivo

SeqA stimulates topoisomerase IV, which relaxes supercoiled DNA and resolves catenated DNA [11]. Optimal concentration of SeqA stimulated topoisomerase IV in the *in vitro* relaxation and decatenation of plasmid DNA [11]. As SeqA concentration increased, aggregation of SeqA on DNA resulted in the aggregation of DNA [11]. Under this condition, SeqA on the substrate plasmids induced topoisomerase IV to catenate plasmids generated by increased intermolecular DNA strand passage. At considerably higher concentration, SeqA coated on DNA and eventually inhibited topoisomerase IV reactions [11]. Therefore, these characteristics were used for testing the aggregation property of SeqA. SeqA(K66E, R70E) more efficiently relaxed supercoiled plasmid DNA than the wild-type protein (Fig. 3A). Although the mutant protein stimulated topoisomerase IV, it was less efficient in generating knotted DNA. Furthermore, this mutant protein did not inhibit topoisomerase IV at the concentration at which wild-type SeqA did (Fig. 3B).

SeqA forms discrete foci that track replication forks [8–10]. We suggested that SeqA foci represented SeqA aggre-

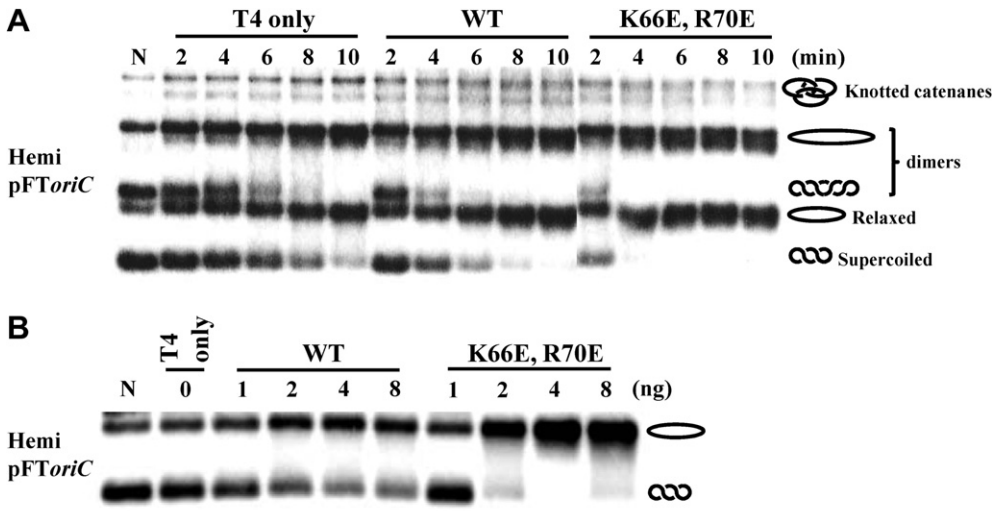

Fig. 3. Mutations in the hinge region reduce the inhibitory effect of SeqA on topoisomerase IV reaction. Topoisomerase IV relaxation assays were performed as described in Materials and methods. (A) Four nanograms of each of the indicated protein was added to the topoisomerase IV reaction mixture (3.8 nM topoisomerase IV and 10 ng (0.5 nM) supercoiled and hemimethylated pBluescript II(+)) followed by incubation at 37 °C for the indicated time. (B) The indicated amount of each protein was incubated for 10 min. The resulting topoisomers were separated on a 1% agarose gel and detected by Southern hybridization with the ^{32}P-labeled PvuII/SacI fragment of pBluescript II(+). The topological states of plasmids in the blot are illustrated on the right side of the autoradiogram. N and T4 only indicated reactions include no protein and only topoisomerase IV, respectively.

gate formed on hemimethylated DNA. Unlike the strains containing wild-type *seqA* gene, WT and *ΔseqA*(+λ*seqA*), the strain, *ΔseqA*(+λ*seqA*(*K66E, R70E*)), which contained mutant *seqA*(*K66E, R70E*) gene on *ΔseqA* background, could not form discrete foci *in vivo* (Fig. 4A). Furthermore, the chromosomes of cells containing *seqA*(*K66E, R70E*) were not evenly segregated similar to *seqA* null mutant and exhibited elongated morphology (Fig. 4B).

The above *in vitro* and *in vivo* results confirmed that the SeqA(K66E, R70E) mutant protein was defective in aggregation on DNA. Therefore, we suggest that positively charged amino acids in the hinge region play a critical role in aggregate formation.

Discussion

We and other researchers have attempted to determine the mechanism of SeqA interactions during hemimethylated DNA binding. A dimeric SeqA molecule binds to a pair of hemi-sites, and the cooperative interactions between SeqA molecules stabilize their DNA binding [12–15]. Previous reports showed that SeqA protein comprises three regions: two lobular domains and the hinge region [12,14,17,18]. The C-domain recognizes and binds to hemi-sites. The smaller N-domain participates in SeqA–SeqA interaction [12,14,17,18]. The AFM image revealed that the SeqA molecule has a dimeric configuration in which two monomers are linked through the N-domain [12]. The N-domain mutants showed weaker affinity to hemimethylated DNA, although their C-domain was intact [12]. Furthermore, the binding specificity was changed; although wild-type SeqA preferred hemi-sites on the same phase of DNA, the mutants hardly discriminated between the relative positions of two hemi-sites [12]. We suggest that the N-domain coordinates the dimeric configuration of SeqA and finely configures the other parts of SeqA to provide optimal binding affinity to a pair of hemi-sites. Thr-18 is located on the N-domain. The SeqA(T18G) mutant protein exhibited defective cooperativity and aggregation (Fig. 2C) [12]. This result suggested that the N-domain participates in the stabilization of SeqA binding through cooperative interactions between DNA bound SeqA molecules.

The hinge region of SeqA that contains repeated positively charged amino acids links the N- and C-domain [12,17]. SeqA loses its aggregation property when the positively charged amino acids in this region were substituted with negatively charged or neutral amino acids (Figs. 1–3). A crystallographic study of SeqA fragment suggested that a part of the hinge region participated in interaction between SeqA fragments [17]. There exists a possibility that this region mediates direct interactions between SeqA proteins for aggregation. Since special arrangement of the hinge region might be critical for SeqA structure, it is also possible that the unique structure of the hinge region, which is supported by a positively charged array, is required for aggregation of SeqA. The hinge region mutants exhibited defects in foci formation and nucleoid segregation (Fig. 4). These results suggest that foci formation and *in vivo* function of SeqA requires aggregation of SeqA, in which the hinge region containing positively charged amino acids participates.

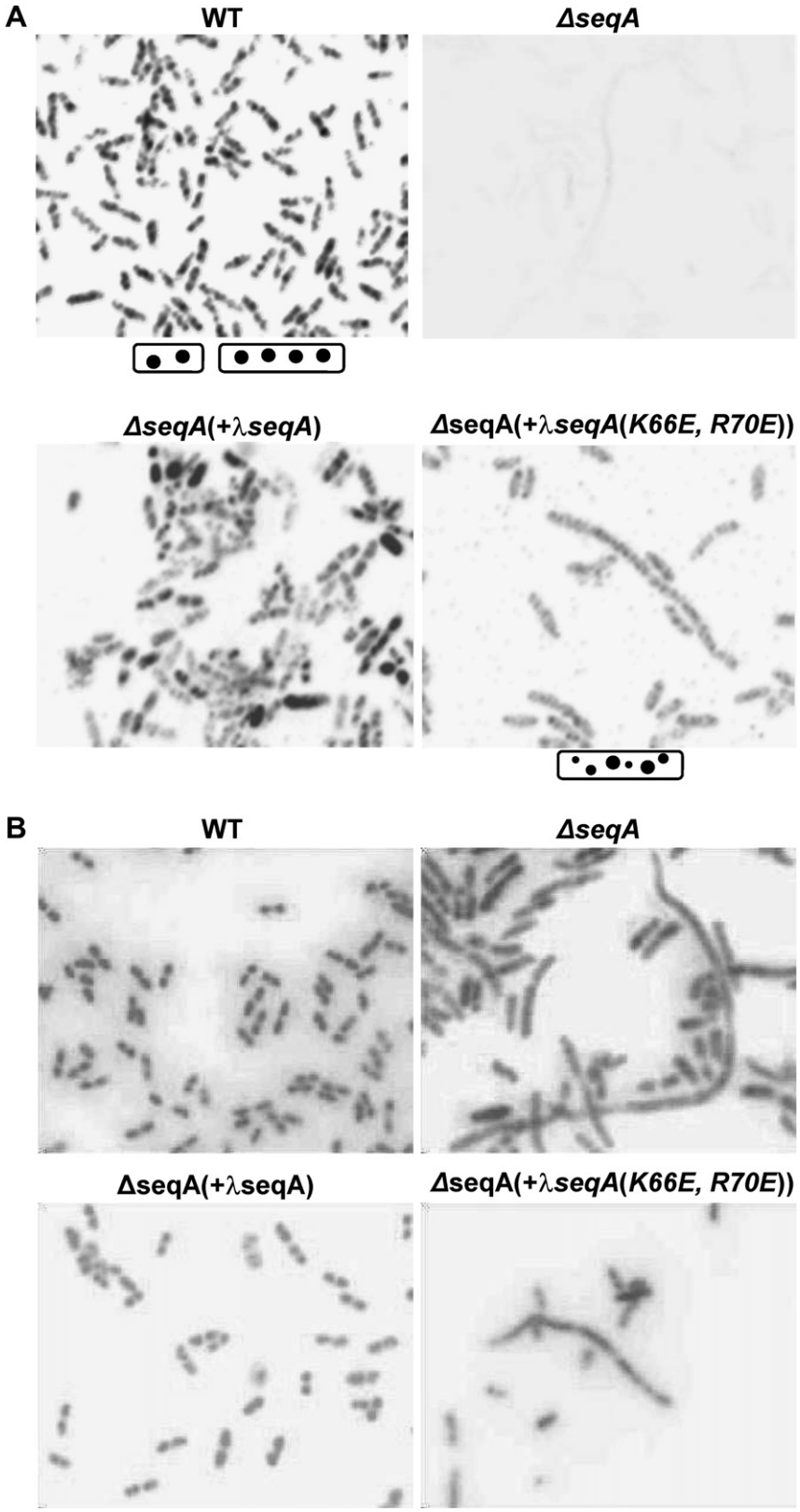

Fig. 4. *seqA(K66E, R70E)* mutant strain was defective in foci formation *in vivo*. SeqA foci formation of wild-type and mutant *seqA* strains was examined. Wild-type W3110 (indicated as WT), *seqA* null strain W3SQT (*seqA*::Tc^R) (*ΔseqA*), and W3SQT harboring lysogenic λ phages expressing wild-type *seqA* or *seqA*(*K66E, R70E*) were immunostained with SeqA antibody and visualized by confocal microscopy (A). The nucleoid of each strain was stained with Hoechst 33258 (B).

Acknowledgments

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References

[1] J.M. Kaguni, A. Kornberg, Replication initiated at the origin (*oriC*) of the *E. coli* chromosome reconstituted with purified enzymes, Cell 38 (1984) 183–190.

[2] A. Kornberg, T. Baker, DNA Replication, Second ed., W.H. Freeman, New York, 1992.

[3] J.L. Campbell, N. Kleckner, *E. coli oriC* and the *dnaA* gene promoter are sequestered from dam methyltransferase following the passage of the chromosomal replication fork, Cell 62 (1990) 967–979.

[4] S. Kang, H. Lee, J.S. Han, D.S. Hwang, Interaction of SeqA and Dam methylase on the hemimethylated origin of *Escherichia coli* chromosomal DNA replication, J. Biol. Chem. 274 (1999) 11463–11468.

[5] S. Wold, E. Boye, S. Slater, N. Kleckner, K. Skarstad, Effects of purified SeqA protein on *oriC*-dependent DNA replication *in vitro*, EMBO J. 17 (1998) 4158–4165.

[6] A. Taghbalout, A. Landoulsi, R. Kern, M. Yamazoe, S. Hiraga, B. Holland, M. Kohiyama, A. Malki, Competition between the replication initiator DnaA and the sequestration factor SeqA for binding to the hemimethylated chromosomal origin of *E. coli in vitro*, Genes Cells 5 (2000) 873–884.

[7] C. Nievera, J.J. Torgue, J.E. Grimwade, A.C. Leonard, SeqA blocking of DnaA-*oriC* interactions ensures staged assembly of the *E. coli* pre-RC, Mol. Cell 24 (2006) 581–592.

[8] S. Hiraga, C. Ichinose, H. Niki, M. Yamazoe, Cell cycle-dependent duplication and bidirectional migration of SeqA-associated DNA–protein complexes in *E. coli*, Mol. Cell 1 (1998) 381–387.

[9] S. Hiraga, C. Ichinose, T. Onogi, H. Niki, M. Yamazoe, Bidirectional migration of SeqA-bound hemimethylated DNA clusters and pairing of *oriC* copies in *Escherichia coli*, Genes Cells 5 (2000) 327–341.

[10] M. Yamazoe, S. Adachi, S. Kanaya, K. Ohsumi, S. Hiraga, Sequential binding of SeqA protein to nascent DNA segments at replication forks in synchronized cultures of *Escherichia coli*, Mol. Microbiol. 55 (2005) 289–298.

[11] S. Kang, J.S. Han, J.H. Park, K. Skarstad, D.S. Hwang, SeqA protein stimulates the relaxing and decatenating activities of topoisomerase IV, J. Biol. Chem. 278 (2003) 48779–48785.

[12] S. Kang, J.S. Han, K.P. Kim, H.Y. Yang, K.Y. Lee, C.B. Hong, D.S. Hwang, Dimeric configuration of SeqA protein bound to a pair of hemi-methylated GATC sequences, Nucleic Acids Res. 33 (2005) 1524–1531.

[13] J.S. Han, S. Kang, S.H. Kim, M.J. Ko, D.S. Hwang, Binding of SeqA protein to hemi-methylated GATC sequences enhances their interaction and aggregation properties, J. Biol. Chem. 279 (2004) 30236–30243.

[14] H. Lee, S. Kang, S.H. Bae, B.S. Choi, D.S. Hwang, SeqA protein aggregation is necessary for SeqA function, J. Biol. Chem. 276 (2001) 34600–34606.

[15] J.S. Han, S. Kang, H. Lee, H.K. Kim, D.S. Hwang, Sequential binding of SeqA to paired hemi-methylated GATC sequences mediates formation of higher order complexes, J. Biol. Chem. 278 (2003) 34983–34989.

[16] R.W. Simons, F. Houman, N. Kleckner, Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions, Gene 53 (1987) 85–96.

[17] A. Guarne, Q. Zhao, R. Ghirlando, W. Yang, Insights into negative modulation of *E. coli* replication initiation from the structure of SeqA-hemimethylated DNA complex, Nat. Struct. Biol. 9 (2002) 839–843.

[18] A. Guarne, T. Brendler, Q. Zhao, R. Ghirlando, S. Austin, W. Yang, Crystal structure of a SeqA-N filament: implications for DNA replication and chromosome organization, EMBO J. 24 (2005) 1502–1511.

[19] N. Fujikawa, H. Kurumizaka, O. Nureki, Y. Tanaka, M. Yamazoe, S. Hiraga, S. Yokoyama, Structural and biochemical analyses of hemimethylated DNA binding by the SeqA protein, Nucleic Acids Res. 32 (2004) 82–92.