SecA defects are accompanied by dysregulation of MukB, DNA gyrase, chromosome partitioning and DNA superhelicity in *Escherichia coli*

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Spatial regulation of nucleoids and chromosome-partitioning proteins is important for proper chromosome partitioning in *Escherichia coli*. However, the underlying molecular mechanisms are unknown. In the present work, we showed that mutation or chemical perturbation of secretory A (SecA), an ATPase component of the membrane protein translocation machinery, SecY, a component of the membrane protein translocation channel and acyl carrier protein P (AcpP), which binds to SecA and MukB, a functional homologue of structural maintenance of chromosomes protein (SMC), resulted in a defect in chromosome partitioning. We further showed that SecA is essential for proper positioning of the *oriC* DNA region, decatenation and maintenance of superhelicity of DNA. Genetic interaction studies revealed that the topological abnormality observed in the secA mutant was due to combined inhibitory effects of defects in MukB, DNA gyrase and Topo IV, suggesting a role for the membrane protein translocation machinery in chromosome partitioning and/or structural maintenance of chromosomes.

INTRODUCTION

The mechanisms of chromosome partitioning in bacterial cells are not known. Currently, two general issues are important in addressing the problem (Fisher et al., 2013). First, how the cell places sister chromosomes in distinct spaces, also named ‘positioning’; and second, the nature of the energy that is required for the process. In this paper, we mainly describe the nature of positioning and the underlying mechanisms involved in the general topology of chromosomes.

In *Escherichia coli*, the replication origin of chromosome (*oriC*) localizes at mid-cell before the onset of replication, and then the replicated sister *oriC* copies move to the 1/4 cellular positions after duplication. Similarly, MukB (‘mukaku’ means anucleate in Japanese), which is a functional homologue of structural maintenance of chromosomes (SMC) protein that mediates chromosome organization, localizes equidistantly along the long cell axis as foci in living cells (Hiraga, 2000; Ohsumi et al., 2001; Adachi et al., 2008); for a review, see Nolivos & Sherratt (2013). In mukB null-deletion (*ΔmukB*) mutant cells, localization of the *oriC* region, nucleoids and replication forks is affected, and these mutant cells exhibit a defect in separation of sister chromosomes at the restrictive temperature (at or above 30 °C); this is referred to as the partition minus (*Par−*) phenotype (Niki et al., 1991). These observations indicate that positioning of these factors through spatial regulation may play important roles in chromosome partitioning (defined as separation of sister chromosomes), but the underlying molecular mechanism of this process is not yet clear.

DNA gyrase and DNA topoisomerase IV (Topo IV), which are involved in topological regulation of DNA, are also critical for chromosome partitioning. Similar to MukB, inactivation of DNA gyrase or Topo IV also results in the Par− phenotype (Wang, 2002). Interestingly, MukB and DNA gyrase functionally interact with each other (Adachi & Hiraga, 2003). MukB and Topo IV also functionally and physically interact (Hayama & Marians, 2010; Li et al., 2010; Hayama et al., 2013). Thus, the topology of the nucleoid or chromosomal region is thought to be important for proper chromosome partitioning in bacteria.

Abbreviations: Cs, cold sensitive; MBP, maltose binding protein; Ts, temperature sensitive.

Supplementary material is available with the online version of this paper.
In *E. coli*, Sun & Margolin (2004) briefly described the Par− phenotype in secretory A (SecA) conditional mutant cells, which they used to address the function of FtsZ, a tubulin homologue. We are interested in the underlying molecular mechanism that causes the chromosome-partitioning defect in the absence of SecA. SecA mediates translocation of membrane proteins through the Sec pore complex of the inner cell membrane, thus maintaining homeostatic conditions in cells via the membrane transport machinery (Robson & Collinson, 2006). SecY is a component of the membrane protein translocation channel together with SecE and SecG (Oliver et al., 1990). SecY and SecB interact with SecA and stimulate the ATPase activity of SecA (Miller et al., 2002; Natale et al., 2004). Therefore, we also investigated the molecular link between SecA and proteins known to be involved in chromosome partitioning such as MukB, DNA gyrase and Topo IV.

In the present work, we demonstrate the roles of SecA–SecY (a component of the membrane protein translocation channel) and of AcpP, which is an interacting partner of SecA, in proper chromosome partitioning.

AcpP is involved in fatty acid biosynthesis, is the sole essential acyl carrier protein in *E. coli* and is acylated during fatty acid biosynthesis as a carrier and donor (De Lay & Cronan, 2006). However, AcpP is also a hub protein in *E. coli* and interacts with MukB as detected with both co-purification (Niki et al., 1992) and high-throughput analysis (Butland et al., 2005). AcpP also interacts with proteins not related to lipid synthesis, such as DNA polymerase III, RNA polymerase and ribosomes, suggesting a variety of functions and the possibility of a role in chromosome partitioning when working together with MukB. Finally, we found functional epistasis of SecA over MukB, DNA gyrase and Topo IV, revealing a novel role for the membrane protein translocation machinery in chromosome partitioning and/or chromosome organization.

**METHODS**

**Bacterial strains and plasmids.** The strains and plasmids used in this study are shown in Table 1 and Table S1 (available in the online Supplementary Material). Derivatives that have the ampicillin-resistant *secA204* mutation were obtained by PI transduction (using PI*vir*) from W208 and were selected and purified with 3–4 mM sodium azide. Derivatives that have the *ΔmukB* mutation were obtained with co-transduction with the kanamycin resistance gene from AZ5372 and were selected and purified with 30 μg kanamycin ml⁻¹. Virulent *E. coli* DL1 (2007), 2005). AcpP also interacts with proteins not related to lipid synthesis, such as DNA polymerase III, RNA polymerase and ribosomes, suggesting a variety of functions and the possibility of a role in chromosome partitioning when working together with MukB. Finally, we found functional epistasis of SecA over MukB, DNA gyrase and Topo IV, revealing a novel role for the membrane protein translocation machinery in chromosome partitioning and/or chromosome organization.

**Analysis of the superhelicity of plasmid DNA molecules with two-dimensional gel electrophoresis.** Two-dimensional gel electrophoresis was performed as previously described (Weitao et al., 2000). Cultures (400 ml) of bacterial strains carrying the plasmid pUC19 were exponentially grown in medium C and harvested at OD₆₅₀mₜ=0.6. Plasmid DNA was extracted using the PureLink HiPure Plasmid Maxiprep kit (Invitrogen) and stored in 0.2 ml TE (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) at −20 °C. The first-dimension run for the horizontal two-dimensional electrophoresis was performed in 0.8 % agarose in Tris-acetate-EDTA (TAE) buffer at 2.5 V cm⁻¹ for 3 h.

**Immunofluorescence microscopy.** Indirect immunofluorescence microscopy using SecA antiserum and goat anti-rabbit IgG antiserum was performed as described previously (Hiraga et al., 1998). We used goat anti-rabbit IgG antiserum conjugated to Cy3 (GE Healthcare) as the secondary antibody.

**Run-off replication method to analyse DNA content per cell using flow cytometry.** This procedure was performed as described previously (Adachi et al., 2008).
Table 1. *Escherichia coli* strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype/description</th>
<th>Resistance</th>
<th>Reference/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>W208</td>
<td><em>thr-1 leuB6(Am) secA204 lacZ4 glyV44(As) rpsL8 thi-1</em></td>
<td>Sm-R, Az-R</td>
<td><em>E. coli</em> genetic stock centre</td>
</tr>
<tr>
<td>IL05 (pLAU53)</td>
<td>AB1157 <em>thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 str-31 tss-33 supE44 tetO60 @ oril1 lacO60 @ ter2</em> (pLAU53)</td>
<td>Ap-R, Km-R, Gm-R</td>
<td>Lau et al. (2003)</td>
</tr>
<tr>
<td>MQ597</td>
<td>IL05 (pLAU53) except secA204</td>
<td>Ap-R, Km-R, Gm-R, Az-R</td>
<td>This study</td>
</tr>
<tr>
<td>MC4100</td>
<td><em>araD139 Δ(argF-lac)U169 rpsL150 relA1 fbbB5301 deoC1 ptsF25 rbS</em></td>
<td>Sm-R</td>
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</tr>
<tr>
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<tr>
<td>C600</td>
<td><em>thi thr leuB lacY tonA supE</em></td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>EJ812</td>
<td>C600 except parC1215 (Ts)</td>
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<td>Yamanaka et al. (1996)</td>
<td></td>
</tr>
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<td><em>leuB6 λ− thyA7 rpsL153 (str) deoC3 dnaT12 (Ts) dnaC2 (Ts)</em></td>
<td>Sm-R</td>
<td>Carl (1970)</td>
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<tr>
<td>MQ756</td>
<td>PC2 except secA204</td>
<td>Az-R, Sm-R</td>
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RESULTS

Sodium azide and mutations in secA, secY and acpP affect the morphology of the nucleoid

Because the secA mutant exhibits the Par− phenotype (Sun & Margolin, 2004), we attempted to recapitulate the Par− phenotype on a sexual basis. When temperature-sensitive (Ts) secA51 mutant cells (MM52) were incubated at the non-permissive temperature of 42 °C in medium L for 4 h, nucleoids in 100% (36/36) of cells were elongated and enlarged, showing the typical Par− phenotype, compared with the isogenic wild-type strain (MC4100) that did not exhibit any abnormalities (0/149 cells, Fig. 1a). This is consistent with a previous report that briefly described the Par− phenotype as a Par− mutant (Sun & Margolin, 2004). We further asked whether inactivation of other components of the membrane protein translocation machinery such as SecY (a membrane protein component of the translocation channel) also resulted in the Par− phenotype. When cold-sensitive (Cs) secY39 mutant cells (AD208) were incubated in medium L at the restrictive temperature of 22 °C for 20 h, 83% (52/63) of cells exhibited the typical Par− phenotype (Fig. 1b). The morphologies of the cells and the nucleoid were normal in both mutants at the permissive temperatures of 30 °C and 37 °C (1/186 and 4/64 abnormalities), respectively. At all temperatures tested, isogenic wild-type strains (MC4100 and TYO) showed normal morphology (1/286 and 4/58 abnormalities).

In addition, we tested a temperature-sensitive acpP mutant (NRD29) and observed that these cells also exhibited the Par− phenotype at a frequency of ~70% (86/125) after incubation at the non-permissive temperature of 42 °C for 3 h (Fig. 1c), but not at the permissive temperature of 30 °C (2/109) or in the isogenic wild-type strain (0/206) (NRD52). The result was similar to that of the ΔmukB mutant at 42 °C (see Introduction).

We next sought to independently recapitulate the Par− phenotype by chemically blocking the ATPase activity of SecA using 1 mM sodium azide (Oliver et al., 1990). Among many essential ATPases in vivo, the SecA ATPase is the sole target of 1 mM sodium azide because an azide-resistant secA204 mutant (MQ597) grew exponentially in the presence of 1 mM sodium azide and grew as well as the mutant in the absence of the chemical (Fig. 1d). On the other hand, the isogenic strain with the wild-type secA allele (IL05/pLAU53) grew exponentially for 2 h after the addition of sodium azide, and then the growth was gradually inhibited (Fig. 1d). Furthermore, the nucleoid...
Fig. 1. Sodium azide, secA and secY mutations cause a chromosome-partitioning defect. Cells were stained with DAPI. Arrowheads indicate elongated cells with large nucleoid(s). White and black arrowheads indicate the cells in which Par− phenotype was observed. (a) Temperature-sensitive secA51 (Ts) mutant (MM52) cells in medium L. A permissive temperature of 30 °C and restrictive temperature of 42 °C were used. (b) Cold-sensitive secY39 (Cs) mutant (AD208) cells in medium L. A permissive temperature of 37 °C and a restrictive temperature of 22 °C were used. (c) Temperature-sensitive acpP D38V (Ts) mutant (NRD29) cells in RB medium. A permissive temperature of 30 °C and a restrictive temperature of 42 °C were used. (d) IL05 (pLAU53) or isogenic secA204 mutant (MQ597) cells were treated with (open circles) or without (solid circles) 1 mM sodium azide at 37 °C for 6 h in medium L. Relative turbidity of cells is shown. (e) Cells of IL05 (pLAU53) growing in medium C at 30 °C were treated with or without 1 mM sodium azide for 3 h. (f) Cells of IL05 (pLAU53) secA204 growing in medium L at 30 °C were treated with or without 1 mM sodium azide for 3 h.
Fig. 2. Sodium azide disrupts positioning of oriC. (a) The proportions of each type of IL05 (pLAU53) cell after the addition of 1 mM sodium azide. Cell types indicated with green shading are 'irregular type (polar type)', in which at least one oriC focus was located in a pole zone. (b) The definition of cell zones. Total cell length is defined as 1. (c) The proportion of 'irregular type (polar type)' cells in IL05 (pLAU53). (d) The proportions of each type of azide-resistant secA204 mutant (MQ597) cell after the addition of sodium azide. (e) The proportion of 'irregular type (polar type)' cells in the azide-resistant secA204 mutant (MQ597). (f) Gel autoradiography of pre-MBP and MBP in the wild-type secA strain IL05 (pLAU53) and the azide-resistant secA204 mutant (MQ597). Lane 1, IL05 (pLAU53); lane 2, IL05 (pLAU53) with 1 mM sodium azide; lane 3, azide-resistant secA204 mutant (MQ597).
Cells of this strain grew exponentially with a doubling time normalized as fluorescent foci by TetR-EYFP (Lau et al., 2005). We further investigated the cause of the chromosome partitioning defect in the secA mutant. Because spatial regulation of nucleoids and chromosome-partitioning proteins is considered to be important for proper chromosome partitioning, we examined the effect of SecA on the positioning of oriC. The oriC region and other chromosomal loci are known to localize as foci, and their intracellular positioning is strictly regulated (Hiraga, 2000), rendering oriC a suitable system for quantitatively detecting positioning defects during chromosome positioning.

We used the IL05 (pLAU53) strain, which contains 240 repeats of the tetO insertion near oriC that can be visualized as fluorescent foci by TetR-EYFP (Lau et al., 2003). Cells of this strain grew exponentially with a doubling time of 150 min in medium C at 30 °C. Samples were taken at various intervals and observed with a fluorescence microscope for oriC foci. According to Bates & Kleckner (2005), the oriC locus localizes at a new pole-biased region at the beginning of the normal cell cycle. The locus then moves to mid-cell, begins replicating, and after a brief period of cohesion, the focus splits in two. One remains at mid-cell, and the other moves towards the 1/4 position in length. Thereafter, the mid-cell focus moves towards the 3/4 position, and each locus remains at its corresponding position until cell division. We normalized the cell length and divided it into five zones as shown in Fig. 2(b). Then, the intracellular localization of oriC foci was classified into 12 types as shown in Fig. 2(a). Because oriC never localizes very close to cell poles in the normal Bates and Kleckner scheme, these 12 types were grouped into two groups, ‘irregular type (polar type)’ (Types 1C, 2E, 2F, 2G, 2H and 2I) and ‘regular type (nonpolar type)’ (the remaining types), depending on whether at least one oriC focus was abnormally localized at a cell pole. Before inhibition of SecA with sodium azide, we found less than 2% of irregular-type cells in the sample. However, irregular-type cells increased to approximately 20% 1 h after the addition of 1 mM sodium azide (Fig. 2a, c).

As shown in Fig. 2(d, e), in the azide-resistant secA204 mutant (MQ597), irregular-type cells were observed at high frequency (approx. 15%), even in the absence of sodium azide. In the membrane translocation assay with MBP, the precursor of MBP (pre-MBP), which was larger than the membrane-translocated mature MBP lacking the signal peptide, was not observed in the secA204 mutant cells grown in either the absence or presence of 1 mM sodium azide (Fig. 2f, lanes 3 and 4). This indicates that the mutant has normal translocation activity for membrane proteins in both growth conditions. This is consistent with previous results reported by Oliver et al. (1990), who showed that the secA204 mutation does not affect SecA ATPase activity or the translocation activity for MBP in vivo. Thus, although the altered SecA protein in the mutant has normal translocation activity for membrane proteins, the protein is defective in its ability to affect oriC positioning.

Involvement of SecA in intracellular positioning of oriC

We further investigated the cause of the chromosome partitioning defect in the secA mutant. Because spatial regulation of nucleoids and chromosome-partitioning proteins is considered to be important for proper chromosome partitioning, we examined the effect of SecA on the positioning of oriC. The oriC region and other chromosomal loci are known to localize as foci, and their intracellular positioning is strictly regulated (Hiraga, 2000), rendering oriC a suitable system for quantitatively detecting positioning defects during chromosome positioning.

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SecA affects the topology of plasmid DNA

Topological regulation of nucleoids or chromosomal regions is considered to be important for proper chromosome partitioning in bacteria because proteins involved in topological regulation of DNA such as MukB, DNA gyrase and Topo IV are critical for partitioning of nucleoids. We asked whether inactivation of SecA resulted in abnormal topology of DNA such as MukB, DNA gyrase and Topo IV functions. For this purpose, we used plasmid assays to monitor the topology of DNA in a series of mutants.

Strains harbouring high-copy pUC19 plasmid were incubated in medium C, and the topology of the plasmids was analysed plasmid topology in the temperature-sensitive secA204 mutant (MQ597); lane 4, azide-resistant secA204 mutant (MQ597) with 1 mM sodium azide. Upper band, pre-MBP; lower band, MBP. IL05 (pLAU53) and the azide-resistant secA204 mutant (MQ597) were incubated with or without 1 mM sodium azide at 30 °C for 10 min before labelling.
secA51 (Ts) mutant (MM52), which exhibits the Par− phenotype. Notably, when the secA51 mutant cells were incubated at the non-permissive temperature of 42 °C for 2 h, covalently closed circular monomer plasmid molecules moved more rapidly (Fig. 3b, b') than those of the wild-type secA strain (MC4100) (Fig. 3a, a') in the second-dimension electrophoresis. This shows that the inactivation of SecA function reduced the superhelicity of plasmid DNA, suggesting inactivation of DNA gyrase, which increases superhelicity of plasmid DNA molecules (Weitao et al., 2000). Furthermore, additional spots were observed that slowly moved in the first-dimension electrophoresis (Fig. 3b, b'). These spots presumably corresponded to plasmid concatemers.

As a control experiment, we examined the effect of inhibiting DNA gyrase on plasmid topology. Novobiocin is primarily an inhibitor of DNA gyrase at a concentration of 100 μg ml⁻¹ in vivo (Adachi & Hiraga, 2003). When cells of the wild-type strain (MC4100) were incubated in the presence of novobiocin (100 μg ml⁻¹) for 6 h, covalently closed circular monomers of plasmid DNA moved more rapidly in the second-dimension electrophoresis (Fig. 3c, c') than those in cells grown without novobiocin treatment (Fig. 3a, a'), indicating the expected reduction in superhelicity. Additional spots presumably corresponding to concatemers moved slowly in the first-dimension electrophoresis. We next examined plasmid molecules in the ΔmukB mutant (MQ761) at the non-permissive temperature of 42 °C for 2 h. As shown in Fig. 3(d, d'), we found that covalently closed circular monomers moved the same as those in the wild-type strain (Fig. 3a, a'), suggesting that the mukB mutation did not markedly affect the superhelicity. However, many additional spots were observed that corresponded to concatemers of plasmid DNA monomers, which moved slowly in the first-dimension electrophoresis.

We speculate that the defects in the SecA function in plasmid topology are synthetic effects due to defects in DNA gyrase and MukB. ΔmukB mutant cells are hypersensitive to novobiocin (Weitao et al., 1999; Onogi et al., 2000). Novobiocin at 100 μg ml⁻¹ is a lethal dose in ΔmukB mutant cells, but not in WT cells (Adachi & Hiraga, 2003). This concentration of novobiocin inhibits separation (decatenation) of sister chromosomes in the ΔmukB mutant. Therefore, we used this concentration to further study the effect of novobiocin in lethality. When ΔmukB mutant cells were treated with 100 μg novobiocin ml⁻¹ for 4 h followed by a shift up to the non-permissive temperature of 42 °C for 2 h, covalently closed circular monomers moved rapidly in the second-dimension electrophoresis (Fig. 3e, e'), similar to the secA51 mutant at the non-permissive temperature (Fig. 3b, b'). A large number and high amount of additional spots corresponding to concatemers were also observed in this condition, the same as the secA51 mutant at the non-permissive temperature.

To confirm that SecA affects the chromosome-partitioning pathway upstream of DNA gyrase and MukB, we tested the secA51 ΔmukB double mutant (MQ760) incubated at the non-permissive temperature of 42 °C both in the presence (Fig. 3f, f') and absence (Fig. 3g, g') of novobiocin. The patterns observed were the same as those observed in the secA51 single mutant (Fig. 3b, b''). This result suggests epistasis of secA over mukB and gyrB (DNA gyrase B subunit gene, target of novobiocin).

Topo IV is known to relax the superhelicity of plasmid DNA molecules and to decatenate DNA concatemers. As a further control, we examined the effect of inactivation of Topo IV on plasmid topology. We used a temperature-sensitive parC1215 mutant (EJ812) with a mutation in the gene encoding the Topo IV subunit ParC. When the parC1215 mutant cells were incubated at the non-permissive temperature of 42 °C for 2 h, covalently closed circular monomer molecules from the mutant cells were detected as three spots that moved more slowly (Fig. 3h, h') than covalently closed circular monomers from wild-type cells (Fig. 3a, a') in the second-dimension electrophoresis as expected, suggesting an increase in negative superhelicity at three different levels. The negative superhelicity of the plasmids in the other spots was also increased, suggesting inactivation of the relaxation activity of Topo IV. In this condition, the amount of plasmid concatamer is less than 10% (Zechiedrich & Cozzarelli, 1995), indicating that very few concatamers are visualized in the gel.

The temperature difference between 42 °C and 30 °C did not significantly affect the pattern of the plasmid topology in the wild-type strain (Fig. 3a, a', i, i'), indicating that the temperature variations used in this study did not affect DNA topology.

**DISCUSSION**

In the present work, we found evidence that links SecA to chromosome-partitioning proteins. (1) The deficiency in SecA, as well as SecY and AcpP, causes a chromosome-partitioning defect. (2) SecA is essential for proper localization of the oriC locus. (3) Deficiency in secA causes alteration of DNA topology via MukB, DNA gyrase and Topo IV. These results demonstrate that SecA, SecY and AcpP are involved in partitioning of the nucleoid. We note that a similar Par− phenotype is observed when the activity of DNA gyrase or Topo IV is inhibited (Wang, 2002), indicating a functional interaction among SecA, SecY and AcpP with DNA gyrase and/or Topo IV.

Inhibition of the ATPase activity of SecA by 1 mM sodium azide caused a defect in proper cellular positioning of oriC and the Par− phenotype. Sodium azide at 1 mM neither significantly inhibited initiation nor ongoing chromosome replication (at least for 1 h); the separation and bidirectional migration of two bidirectional-replication forks to cell 1/4 positions from mid-cell (Hiraga et al., 2000; Onogi et al., 2002) were also not affected (Fig. S2). The cells with the wild-type secA allele grew exponentially for at least 2 h after the addition of 1 mM sodium azide (Fig. 1d). Thus,
**Fig. 3.** Two-dimensional gel electrophoresis of pUC19 plasmids shows secA activity that is related to DNA topology via MukB, DNA gyrase, and/or Topo IV. (a–f) Photographs of two-dimensional gel electrophoresis. Black and white photo images are reversed in the figure. (a) Wild-type cells (MC4100) at 30 °C. (b) secA51 (Ts) mutant cells (MM52) incubated at the non-permissive temperature of 42 °C for 2 h. (c) Wild-type cells (MC4100) treated with 100 μg ml⁻¹ novobiocin for 6 h. (d) ΔmukB mutant cells (MQ761). (e) ΔmukB mutant cells (MQ761) treated with 100 μg ml⁻¹ novobiocin for 6 h. (f) secA51 ΔmukB double mutant cells (MQ760) treated with 100 μg ml⁻¹ novobiocin for 6 h. (g) secA51 ΔmukB double mutant cells (MQ760) without novobiocin treatment. (h) parC1215 (Ts) mutant cells (EJ812) incubated at the non-permissive temperature of 42 °C for 2 h. (i) Wild-type cells (MC4100) at 42 °C. (a–i) Schematic interpretations of photographs. w, sample wells; g, contaminating genomic DNA segments. 1ccc, covalently closed circular monomers of the plasmid DNA. Dotted lines indicate the gel position corresponding to closed circular monomers in the WT strain (a).
the chemical affected cell growth after a lengthy time. The Par− phenotype observed in these secA, secY and acpP mutants at the non-permissive temperatures may be due to a defect in decatenation of sister chromosomes, as supported by the two-dimensional gel electrophoresis of plasmid DNA (Fig. 3).

The secA51 (Ts) mutation caused both catenation and a reduction in the supercoiling of plasmids at the non-permissive temperature. This result suggests that the wild-type SecA protein is involved in the control of decatenation and superhelicity of plasmid DNA molecules. We postulate that the effects of SecA deficiency on the superhelicity of the plasmids and the partitioning of nucleoids are achieved via the functions of MukB, DNA gyrase and possibly Topo IV. DNA gyrase has been suggested to decatenate circular DNA molecules only in the presence of Topo IV (relaxing superhelicity). This is also consistent of both DNA gyrase (promoting negative superhelicity) and Topo IV (relaxing superhelicity). This result suggests that the wild-type SecA protein is involved in the control of decatenation and superhelicity of plasmid DNA molecules only in the presence of Topo IV in vivo (Zechiedrich & Cozzarelli, 1995). MukB likely forms DNA topology suitable for decatenation, and the absence of mukB results in DNA decatenation defects, possibly through this mechanism. In the ΔmukB mutant, superhelicity was still apparently normal, which may be due to weaker activities of both DNA gyrase (promoting negative superhelicity) and Topo IV (relaxing superhelicity). This is also consistent with the result that the secA51 single mutant at the non-permissive temperature, regardless of the presence of novobiocin (Fig. 3b, b'), exhibited plasmids with the same topology as the ΔmukB single mutant with novobiocin (Fig. 3c, e') or the double secA51 and ΔmukB double mutant both in the presence and absence of novobiocin (Fig. 3f, f', g, g'). SecA may be involved in the subcellular physiological regulation of MukB, DNA gyrase, and possibly Topo IV.

Inactivation of SecA may disrupt the translocation of membrane transporter proteins, resulting in abnormal intracellular physiology such as accumulation of membrane protein precursors or abnormal ionic conditions in which MukB, DNA gyrase and Topo IV are inactivated. However, we speculate that this hypothesis is unlikely according to the following observations. First, as mentioned later, our findings are supported by protein–protein interactions. Second, the secA204 mutant has a defect in the proper positioning of oriC foci, whereas membrane protein translocation activity is normal (Fig. 2f). The secA204 mutant may be a mutant of oriC positioning although the translocation activity is normal. Note that the effect of the secA204 mutation on oriC positioning is not due to inhibition of SecA ATPase activity (Fig. 2f, Oliver et al., 1990). We suggest that the altered SecA protein in the secA204 mutant may fail to interact with unknown proteins that are involved in the mechanism of oriC positioning, such as the migS-dependent oriC positioning mechanism (Yamaichi & Niki, 2004). Certainly, additional effects on chromosome partitioning in secA may exist that are more important than oriC positioning. A comparison of the severe defects in secA51 mutants and the mild defect in secA204 mutants suggests the possible involvement of the essential AcpP.

Our current findings are further supported by protein–protein interactions, suggesting the existence of a chromosome-partitioning protein network centralized by SecA. Chromosome-partitioning proteins such as MukB, Topo IV and DNA gyrase appear to physically interact with SecA, SecY and AcpP, among approximately 4300 proteins in E. coli (Butland et al., 2005). The result of the large-scale protein–protein interaction network suggests the presence of a subnetwork of Topo IV-MukB-AcpP-SecA, inferring a possible functional link among these proteins. In addition, AcpP copurifies with MukB (Niki et al., 1992). Furthermore, SeqA (Sequestration A) protein, which binds to hemimethylated nascent DNA segments (Hiraga, 2000; Yamazoe et al., 2005), interacts with ParC (Kang et al., 2003). Finally, stimulation of Topo IV activity by MukB and execution of MukB activity through Topo IV-MukB interactions have been shown in previous studies (Hayama & Marians, 2010; Li et al., 2010; Hayama et al., 2013). Fig. 4 shows a schematic of the physical interactions among these proteins and their relationships to the mechanism of chromosome partitioning, chromosome structural maintenance and oriC positioning.

Our recent results (associated, submitted manuscript) suggest that this SecA-mediated mechanism acting together with DNA-binding proteins is an important candidate for

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**Fig. 4.** Protein–protein interaction map of proteins described in this manuscript. Interactions were deduced from Niki et al. (1992); Kang et al. (2003); Natale et al. (2004); Butland et al. (2005); Hayama & Marians (2010); Li et al. (2010); and Hayama et al. (2013). The large-scale interactions based on Butland et al. (2005) are indicated as short broken lines, and all other known interactions are indicated as solid lines. Long broken lines show that SecA is involved in the oriC positioning mechanism.
molecular ‘tethering’ during segregation of sister chromosomes and shows the role of bacteria in the emerging field that unifies physics and biology on a micrometer scale.

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