

Contribution of SMC (Structural Maintenance of Chromosomes) and SpoIIIE to Chromosome Segregation in Staphylococci[∇]

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In contrast to rod-shaped bacteria, little is known about chromosomal maintenance and segregation in the spherical *Staphylococcus aureus*. The analysis of chromosomal segregation in *smc* (structural maintenance of chromosomes) and *spoIIIE* single and double mutants unravels differences in the chromosome dynamics in the spherical staphylococcal cells compared to the model in rods.

In bacteria, studies on chromosome dynamics are mainly focused on model organisms such as *Escherichia coli*, *Bacillus subtilis*, and *Caulobacter crescentus* (8, 28). However, in staphylococci, with their spherical cell shape and their special consecutive perpendicular mode of division (34), little is known about chromosome organization and dynamics so far. Moreover, the important status of *Staphylococcus aureus* as one of the most prominent human pathogens highlights the significance of studying essential cellular processes that could serve as a basis for antibiotic target development (11, 15).

SMC (structure maintenance of chromosomes) protein is one of the crucial factors involved in various aspects of chromosome dynamics, such as chromosome condensation, packaging, partitioning, and DNA repair (4, 6, 13, 22, 24, 30). *B. subtilis* SMC and its homolog, MukB, in *E. coli* are composed of two head regions at N and C termini, setting up an ATPase domain separated by two heptad-rich regions forming a single internal long coiled coil that are connected by a flexible hinge domain in the middle. Homodimerized SMC proteins linked by the hinge domain (17) form a complex with ScpA and ScpB (segregation and condensation proteins A and B) (23), while MukB interacts with MukF and MukE in *E. coli* correspondingly (27). The *B. subtilis smc* mutant shows a severe temperature-sensitive lethal phenotype with irregular chromosome organization and chromosome segregation defects (4, 24). It was proposed that the pleiotropic phenotype of the *smc* mutant was primarily due to its function in chromosome condensation (4). Interestingly, a recent report described that the mycobacterial *smc* deletion mutant was still proficient in DNA repair and long-term survival (14).

The involvement of SpoIIIE in chromosome segregation was first identified during sporulation in *B. subtilis* (32). It is required for active translocation of the bulk chromosome into the forespore across the fused septal membranes (5). SpoIIIE consists of an N-terminal transmembrane domain responsible for membrane anchoring and the C-terminal ATPase and DNA translocation domain. The function of SpoIIIE in post-

septational chromosome partitioning renders it as a backup mechanism to rescue the nucleoids that have been trapped by the division septum when chromosome segregation was perturbed in vegetative cells (26). Consequently, the *smc* and *spoIIIE* double mutant had a synergistic lethal phenotype in *B. subtilis* (3). The combination of the *E. coli mukB* null mutation and truncation of *ftsK* encoding the homolog protein to SpoIIIE resulted in a similar synergistic lethal phenotype (33).

Genome analysis revealed that there was a single *smc* gene locus present in all of the staphylococcal genomes available so far. *S. aureus smc* (SAOUHSC_01204) encodes a 1,188-amino-acid polypeptide with a calculated molecular mass of 136.7 kDa that shares 42.8% similarity with *B. subtilis* SMC and consists of the typical domain structures of the SMC protein family analyzed by ClustalW2 alignment and SMART (Simple Modular Architecture Research Tool). During our previous work, a transposon Tn917 mutagenesis library was constructed in an *S. aureus* double-knockout strain (DKO1) in which two genes encoding “lipopolysaccharide modification acyltransferase” and “acyltransferase” were deleted (16). One insertion mutant (DKO1.6) was identified that carried Tn917 at nucleotides 1078 to 1087 within the *smc* gene. In order to study the function of SMC involved in chromosome organization and segregation, *smc::Tn917* was phage transduced into wild-type (WT) *S. aureus* strain SA113, generating a single *smc::Tn917* mutant (Fig. 1A). Phage transductions were performed at 23°C to decrease the probability of suppressor mutations. Isolated mutants were confirmed by sequencing. Surprisingly, SA113 *smc::Tn917* showed similar growth behavior to WT cells both on tryptic soy agar (TSA) plates and in tryptic soy broth (TSB) liquid medium at all three temperatures (30°C, 37°C, and 42°C) tested (Fig. 2A and B). These observations were in contrast to the previous findings in *B. subtilis*. The *B. subtilis Δsmc* mutant was temperature sensitive, the mutation was lethal in rich medium, and the mutant could only grow at 23°C in this medium (4, 24).

Next, we sought to verify if the loss of functional SMC would affect chromosome segregation in *S. aureus*. Samples were taken from exponentially growing cultures at three temperatures for fluorescent microscopy examination (Leica DM5500B). DNA was stained with DAPI (4',6-diamidino-2-phenylindole), the cell membrane was labeled with FM1-43,

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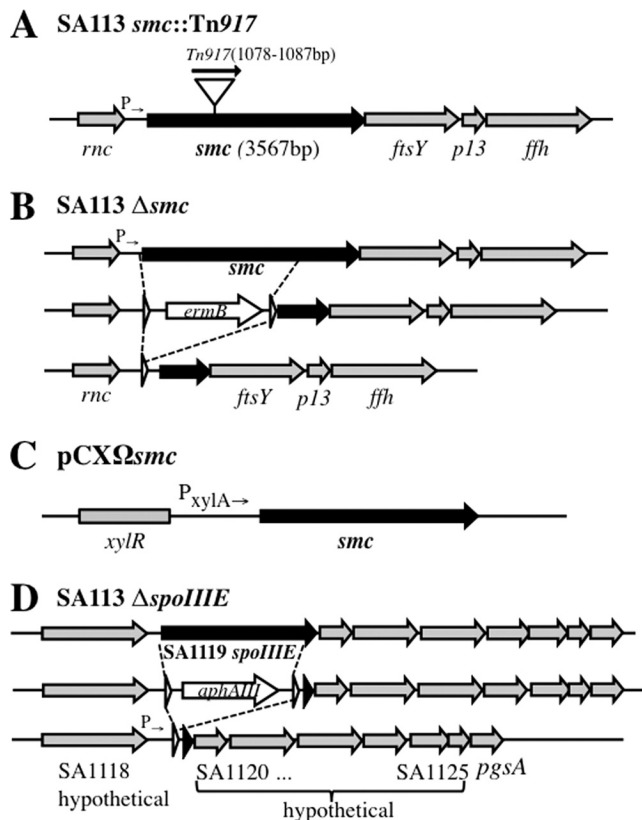


FIG. 1. (A) *Tn917* insertion site in SA113 *smc::Tn917*. (B) Construction of SA113 Δsmc . The *smc* coding sequence, except for 1,294 bp at the 3' end, was replaced by *ermB* cassette flanked with *lox* sites that was further removed by Cre recombinase. (C) Scheme of $pCX\Omega smc$. The *smc* gene was cloned into $pCX15$ (31), under the transcriptional control of the xylose promoter/operator system. (D) Construction of SA113 $\Delta spoIII E$. The *spoIII E* coding sequence, except for 68 bp at the 3' end, was replaced by an *aphAIII* cassette flanked with *lox* sites and further removed by Cre recombinase; white arrowheads represent *lox* sites.

and the cell wall was labeled with BODIPY (boron-dipyrromethene)-vancomycin (Van-FL) to visualize different cellular structures. While in the rod-shaped cells of *E. coli* and *B. subtilis*, the chromosome seems to occupy approximately three-fourths of the cell volume (4, 24), in *S. aureus* the chromosome almost fills the entire cell compartment, as demonstrated by DAPI staining (Fig. 3A). In SA113 *smc::Tn917*, about 10% of the cells were devoid of nucleoids; they appeared as anucleate cells (“black cells” stained with DAPI) that indicated defects in chromosome segregation (Fig. 3B). The anucleate cells were also observed in SA113-DKO1.6 that carried *smc::Tn917* but not in SA113-DKO1, confirming that the chromosome segregation defect was due to the mutation in the *smc* gene. In SA113 *smc::Tn917*, “half-black cells” were observed (2% of 911 cells counted at 30°C) which were composed of one anucleate hemisphere and one normal hemisphere with regular chromosome content and morphology (Fig. 3B, arrow). We assume that this kind of “half-black cell” would be able to further divide into one anucleate daughter cell and one normal daughter cell as the cross wall could correctly form in the middle of the cell (Fig. 3B; staining with Van-FL [green]) and single spherical anucleate cells were observed frequently (Table 1). Furthermore, anucleate diplococci (Fig. 4B, yellow arrow) were observed, although at a low rate of 0.12% (2 pairs of black diplococci out of 1,634 cells counted). It is yet difficult to distinguish whether these anucleate diplococci were divided from one anucleate cell or were divided from two “half-black cells” and afterwards detached from tetrads composing two normal cells and these two anucleate cells. Intriguingly, quantitative analysis revealed that the percentage of anucleate cells decreased by 3-fold at 42°C in the *smc::Tn917* strain (Table 1), suggesting that the defect of chromosome segregation caused by SMC mutation could be relieved by higher growth rate, probably due to the accelerated

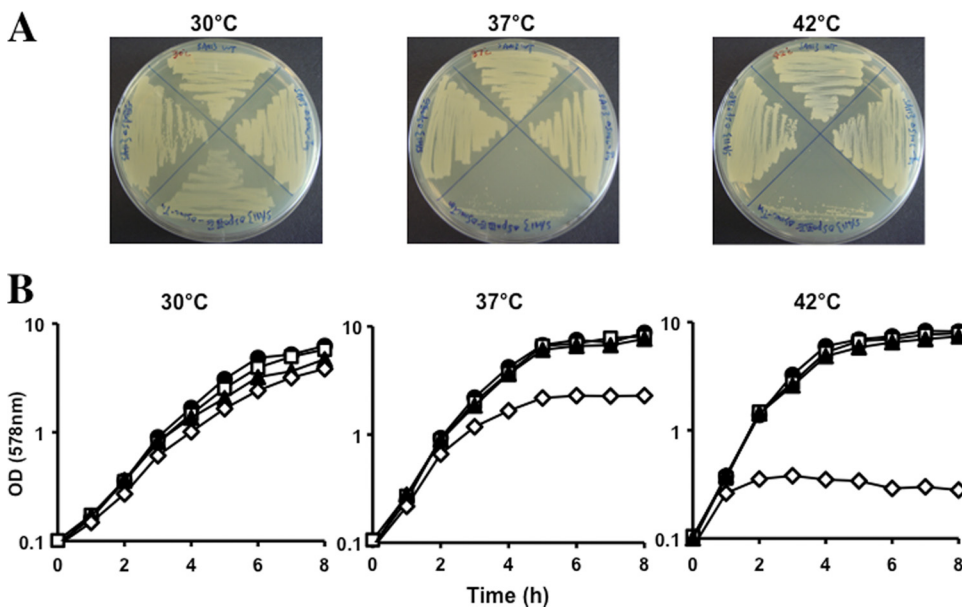


FIG. 2. (A) Growth comparison of the SA113 WT (top) and *smc::Tn917* (right), $\Delta spoIII E$ (left), and *smc::Tn917* $\Delta spoIII E$ (bottom) mutants on TSB agar plates at 30°C, 37°C, and 42°C for 24 h. (B) Growth in TSB rich medium. ●, WT; □, *smc::Tn917* mutant; ◇, $\Delta spoIII E$ mutant; ○, *smc::Tn917* $\Delta spoIII E$ mutant.

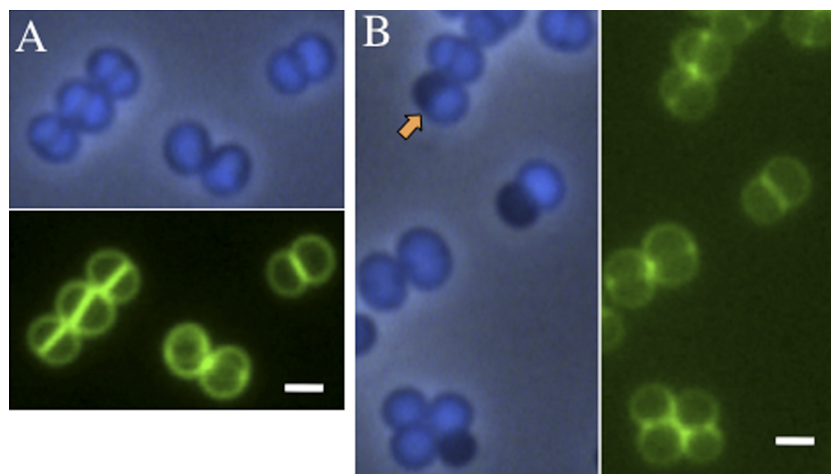


FIG. 3. Chromosome distribution in the SA113 WT and SA113 *smc::Tn917* mutant from mid-log-phase TSB cultures at 30°C. (A) SA113 WT. Newly formed equatorial rings could be visualized in two tilted cells. (B) SA113 *smc::Tn917*. The orange arrow indicates the “half-black cell.” The cell wall was stained with Van-FL (green), and the chromosome was stained with DAPI (blue) and visualized in the phase-contrast–fluorescent merging mode. Scale bar, 1 μ m.

chromosome replication which was assumed to be one of the driving forces for chromosome segregation (7). Notably, no other aberrant chromosome distribution or abnormal cell shape could be found in *S. aureus smc::Tn917* mutants, except for the anucleate cells (Table 1). Thus, SMC’s function as condensin appeared to be less critical in *S. aureus* in contrast to *B. subtilis* SMC or *E. coli* MukB. Furthermore, the chromosome segregation defect in the *S. aureus smc::Tn917* strain could be partially complemented by plasmid-encoded SMC,

which is controlled by xylose inducible promoter (pCX Ω *smc*) (31) (Fig. 1C and Table 1).

In the genome of *S. aureus*, *smc* is located directly upstream of *ftsY*, *p13*, and *ffh*, the genes encoding components of the signal recognition particle protein translocation system. In order to rule out the possible polar effect of *Tn917* on the downstream genes’ expression, a markerless partial deletion *smc* mutant was constructed (Fig. 1B). A 1.3-kb fragment of *smc* gene at its 3’ was left intact due to the consideration that it

TABLE 1. Quantitative analysis of irregular chromosome appearance from mid-log-phase culture^a

Temp and strain genotype	Total no. of cells counted	% of cells:				
		Anucleate	With “CUT” phenotype	With uneven chromosome distribution	With increased/decreased DNA content	Big/small ^b
30°C						
WT	911	0.3	NO ^c	NO	0.2/NO	NO
Δ <i>smc</i>	1,258	9.4	NO	0.3	0.3/NO	NO
<i>smc::Tn917</i>	881	10.4	NO	0.3	0.3/NO	NO
<i>smc::Tn917</i> pCX Ω <i>smc</i>	1,316	2.3	NO	0.5	0.3/NO	0.1/NO
Δ <i>spoIIIE</i>	975	0.7	NO	0.4	0.5/NO	NO/0.1
<i>smc::Tn917</i> Δ <i>spoIIIE</i>	802	4.8	1.9	10.7	3.2/6.1	1.6/4.5
37°C						
WT	1,023	0.3	NO	0.7	0.6/NO	NO
Δ <i>smc</i>	1,144	7.9	NO	0.7	0.5/NO	NO/0.3
<i>smc::Tn917</i>	851	7.2	NO	0.8	0.7/0.2	NO/0.2
<i>smc::Tn917</i> pCX Ω <i>smc</i>	1,216	1.8	NO	0.7	0.7/0.3	NO/0.3
Δ <i>spoIIIE</i>	732	0.4	NO	0.7	0.8/NO	NO/0.1
<i>smc::Tn917</i> Δ <i>spoIIIE</i>	826	6.4	1.7	20.8	5.3/6.2	2.9/4.5
42°C						
WT	987	0.3	NO	1.5	1.5/0.8	NO/0.7
Δ <i>smc</i>	1,240	2.5	NO	1.4	1.8/1.0	NO/0.9
<i>smc::Tn917</i>	922	3.5	NO	1.5	2.0/0.9	NO/1.1
<i>smc::Tn917</i> pCX Ω <i>smc</i>	1,232	1.7	NO	1.5	1.9/1.2	0.3/1.1
Δ <i>spoIIIE</i>	890	0.3	NO	1.6	2.1/1.0	NO/0.9
<i>smc::Tn917</i> Δ <i>spoIIIE</i>	681	6.2	2.0	28.5	5.3/11.2	4.0/7.0

^a Data were summarized from three independent experiments.

^b Big cells are ≥ 1.5 μ m in diameter, and small cells are ≤ 0.5 μ m in diameter.

^c NO, not observed within the total number of cells counted.

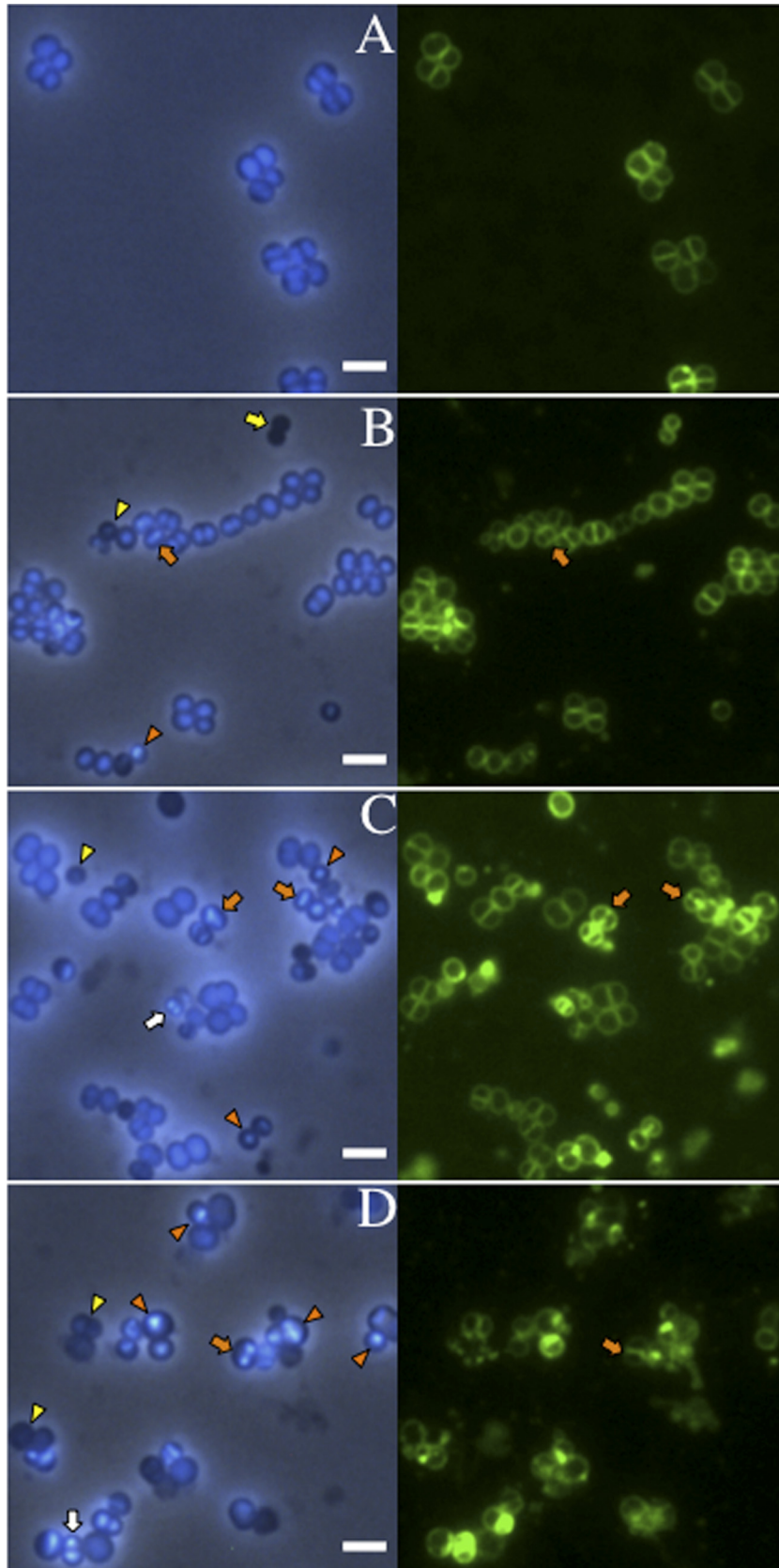


FIG. 4. Altered chromosome morphology aggravated at higher temperatures in SA113 *smc::Tn917 ΔspoIIIIE*. (A) SA113 WT grown for 1.5 h at 42°C. (B to D) SA113 *smc::Tn917 ΔspoIIIIE* grown for 1.5 h after dilution of the overnight culture into fresh TSB medium at 30°C, 37°C, and 42°C respectively. DNA was stained with DAPI (blue), and the cell membrane was labeled with FM1-43 (green). Orange arrows indicate examples of the “guillotine effect,” or ‘CUT’ phenotype; yellow arrows indicate anucleate diplococci; white arrows indicate increased DNA content; yellow arrowheads indicate decreased DNA content; and orange arrowheads show the unevenly distributed nucleoid. Scale bar, 2 μm.

contains a potential promoter controlling the downstream genes' expression. SA113 Δsmc exhibited the same growth curve as SA113 $smc::Tn917$ (data not shown) and a similar rate of chromosome segregation deficiency (Table 1).

Why is the effect of *smc* mutation in staphylococci less severe than that in rod-shaped bacteria? First, the discrepancy could be explained partially by the morphological difference between rods and cocci. Given that *E. coli* or *B. subtilis* cells are 1.1 to 1.5 μm wide and 2.0 to 6.0 μm long, the staphylococcal cell is only half the size (0.5 to 1.5 μm in diameter) of a bacillus cell (12). Besides, *S. aureus* has a much smaller cell volume of 0.15 μm^3 compared to *E. coli* with 0.5 to 0.7 μm^3 (19, 25). Despite different genome sizes between *B. subtilis* and *S. aureus* (4.1 Mbp versus 2.9 Mbp, respectively) (20, 29), the small staphylococcal cell size becomes spatially limiting for the nucleoids; therefore, decondensed chromosomes were not observed in *S. aureus smc* mutant cells. Second, as chromosome segregation is one of the most important cellular processes, bacteria must have employed redundant mechanisms. Other factors may play a more important role during chromosome segregation in the spherical cells.

Earlier studies showed that the DNA translocase SpoIIIE could rescue the trapped chromosome from septum membrane during vegetative growth in *B. subtilis* (26). In order to evaluate the function of SpoIIIE in *S. aureus*, an SA113 *spoIIIE* deletion mutant was constructed via double-cross homologous recombination by using a counterselection vector, pKOR1 (1). A majority of the *spoIIIE* reading frame, except for 68 bp at the 3' end (which contains the Shine-Dalgarno sequence of the downstream gene), was replaced with an *aphAIII* cassette that renders kanamycin resistance (Fig. 1D). The *aphAIII* cassette was flanked with *lox* sites and was further removed by Cre recombinase (21). Afterwards, $smc::Tn917$ was transduced into SA113 $\Delta spoIIIE$, resulting in the SA113 *smc spoIIIE* double mutant. Consistent with previous findings in *B. subtilis* (26), *S. aureus spoIIIE* single mutant cells had neither a significant growth disadvantage (Fig. 2A and B) nor an obvious defect in chromosome distribution compared to the WT (data not shown).

However, distinct from *B. subtilis*, in *S. aureus* a viable *smc spoIIIE* double mutant could be isolated. Growth curves showed that the SA113 *smc spoIIIE* double mutants were temperature sensitive (Fig. 2A and B). Colonies can only be formed at low temperatures on TSA plates. In liquid medium, the SA113 *smc spoIIIE* double mutant could grow to an optical density at 578 nm (OD_{578}) of 2.2 after 8 h at 30°C, whereas the growth ceased after only two generations (growing for 2 h to an OD_{578} of 0.4) at 42°C. Shifting the culture from 24°C to 42°C resulted in rapid growth cessation. To examine the morphological changes in the SA113 *smc spoIIIE* double mutant, samples were taken at early (1.5 h) and late (5 h) growth phases after dilution of overnight cultures into fresh TSB medium and incubated at various temperatures. At the early growth phase, the SA113 *smc spoIIIE* double mutant had distorted chromosome organization and heterogeneous cell sizes, as depicted in Fig. 4B to D and quantified in Table 1. Approximately 2% of the cells showed the so-called "guillotine effect" or "CUT" phenotype that occurred when the chromosome was bisected by the septal membrane (Fig. 4, orange arrows; Table 1), which was not observed in WT cells or *smc* single mutants. The

percentage could not be exactly counted due to the limitations of the two-dimensional microscopy technique. Four to 6% of the double mutant cells were anucleate, which did not increase significantly compared to the *smc* single mutant (Table 1). While the same staining method was always applied, 3-fold more mutant cells appeared to contain an increased amount of DNA compared to WT (bright blue cells in Fig. 4C and D [white arrows]). More significantly, about 11% of the mutant cells showed decreased content of DNA (Fig. 4, yellow arrowheads), which was 10 fold higher than WT or the single mutants. The most severe irregular morphology is the unevenly distributed chromosomes that were no more homogeneous but rather accumulated punctately in nearly 30% of the mutant cells (Fig. 4, orange arrowheads). These aberrant chromosomal morphological changes suggested that the *smc spoIIIE* double mutant was greatly deficient not only in chromosome segregation but also in chromosome structure maintenance, and the defect in chromosome structure appeared even more severe than the segregation defect. The SA113 *smc spoIIIE* double mutant also had heterogeneous cell sizes; cells appeared as big ($\geq 1.5 \mu\text{m}$ in diameter) as well as small ($\leq 0.5 \mu\text{m}$ in diameter) (Table 1). The heterogeneous cell sizes are probably an indirect consequence of the *smc spoIIIE* mutations, since (i) there is no significant correlation between cell size change and the degree of chromosomal disorder (Table 1) and (ii) the defect in chromosome structure and segregation may interfere with many other cellular processes that affect cell size. At the late growth stage, the chromosome structure was severely disrupted, the cells eventually lysed, and more cell debris was produced at 42°C (data not shown). However, at 30°C, although the irregular chromosome disorder was aggravated and the cell size differentiated more obviously than during the earlier growth phase, there were still considerable amounts of normal cells with regular chromosome structure (data not shown).

Thus, compared to SA113 *smc* and *spoIIIE* single mutants, the highly distorted chromosome arrangement in *S. aureus smc spoIIIE* double mutants indicated that SpoIIIE compensated for SMC's function in chromosome segregation to a certain extent. The aggravated chromosome segregation defect in the *smc spoIIIE* double mutant disclosed the chromosome maintenance deficiency in the *smc* single mutant, since it is unlikely that SpoIIIE had a direct role in chromosome organization. In other words, the optimal chromosome segregation facilitates proper chromosome organization in *S. aureus*. While the number of anucleate cells decreased with increased temperature in the *smc* single mutant, the effect was reversed in the *smc spoIIIE* double mutant, where the number of anucleate cells was slightly increased at higher temperature. Interestingly, earlier transcriptomic data for the heat shock response in *S. aureus* showed that *spoIIIE* was transcribed 2.15-fold higher at 48°C (9). This finding could explain that the defect in the *smc* single mutant is in part compensated for by overexpression of SpoIIIE. Distinct from *B. subtilis* or *E. coli*, *S. aureus smc spoIIIE* double mutants are viable at low temperature, although possible suppressor mutations could not be excluded. The distinction underlies a different mechanism for guaranteeing maximum chromosome segregation in staphylococci. Presumably, besides the difference in cell shape,

other factors that play more significant roles in chromosome partitioning in cocci than in rods need to be identified.

Recently it has been found in *B. subtilis* that a second FtsK/SpoIIIE-like protein, SftA (septum-associated FtsK-like translocase of DNA), coordinates chromosome translocation to ensure maximum chromosome segregation, however, at a different stage of cell division from SpoIIIE (2, 18). SftA contains the C-terminal DNA binding and ATPase domain, but instead of the N-terminal transmembrane domain in SpoIIIE, it has a soluble domain. SftA translocates chromosomes before septation, while SpoIIIE comes into play postseptationally when chromosomes are trapped by the septal membrane. Like *B. subtilis spoIIIE* mutants, the *sftA* mutants had a synergistic lethal defect with an *smc* deletion (18). The *B. subtilis sftA spoIIIE* double mutant undergoes normal growth, while the defect in chromosome segregation is exacerbated significantly compared to that in both single mutants. A protein homologous to *B. subtilis* SftA with conserved domain structures is encoded in all staphylococcal genomes, and the homologs share 33.3% identity. It is highly interesting to evaluate the function of SftA in staphylococci for future investigation.

Another aspect that should be considered about staphylococcal chromosome segregation is the specific cell division mode of staphylococci. In contrast to *B. subtilis* or *E. coli*, where division always takes place in the middle of the longitudinal cell, in *S. aureus* the division plane shifts by 90° to the previous one (10). Accordingly, the chromosome replication and segregation that occur prior to cell division must occur perpendicularly as well. Theoretically, in the spherical cell, there are infinite numbers of future division planes that are perpendicular to the previous division disc. The flexibility in shifting the orientation of chromosome replication and segregation might allow staphylococci to cope with the deficiency of chromosome segregation in *smc spoIIIE* double mutants. The regulation of setting the division plane and chromosome segregation direction in staphylococci is totally unknown yet.

Taken together, we have obtained a first insight into chromosome segregation in *Staphylococcus* and presented intriguing results regarding the genetic interaction of SMC and SpoIIIE, two critical factors involved in chromosomal dynamics. These findings shed light on different mechanisms of chromosome dynamics in the spherical staphylococcal cells, in contrast to the model in rods, which definitely deserves future investigation.

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REFERENCES

- Bae, T., and O. Schneewind. 2006. Allelic replacement in *Staphylococcus aureus* with inducible counter-selection. *Plasmid* 55:58–63.
- Biller, S. J., and W. F. Burkholder. 2009. The *Bacillus subtilis* SftA (YtpS) and SpoIIIE DNA translocases play distinct roles in growing cells to ensure faithful chromosome partitioning. *Mol. Microbiol.* 74:790–809.
- Britton, R. A., and A. D. Grossman. 1999. Synthetic lethal phenotypes caused by mutations affecting chromosome partitioning in *Bacillus subtilis*. *J. Bacteriol.* 181:5860–5864.
- Britton, R. A., D. C. Lin, and A. D. Grossman. 1998. Characterization of a prokaryotic SMC protein involved in chromosome partitioning. *Genes Dev.* 12:1254–1259.
- Burton, B. M., K. A. Marquis, N. L. Sullivan, T. A. Rapoport, and D. Z. Rudner. 2007. The ATPase SpoIIIE transports DNA across fused septal membranes during sporulation in *Bacillus subtilis*. *Cell* 131:1301–1312.
- Dervyn, E., M. F. Noirot-Gros, P. Mervelet, S. McGovern, S. D. Ehrlich, P. Polard, and P. Noirot. 2004. The bacterial condensin/cohesin-like protein complex acts in DNA repair and regulation of gene expression. *Mol. Microbiol.* 51:1629–1640.
- Draper, G. C., and J. W. Gober. 2002. Bacterial chromosome segregation. *Annu. Rev. Microbiol.* 56:567–597.
- Errington, J., R. A. Daniel, and D. J. Scheffers. 2003. Cytokinesis in bacteria. *Microbiol. Mol. Biol. Rev.* 67:52–65.
- Fleury, B., W. L. Kelley, D. Lew, F. Gotz, R. A. Proctor, and P. Vaudaux. 2009. Transcriptomic and metabolic responses of *Staphylococcus aureus* exposed to supra-physiological temperatures. *BMC Microbiol.* 9:76.
- Giesbrecht, P., T. Kersten, H. Maidhof, and J. Wecke. 1998. Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin. *Microbiol. Mol. Biol. Rev.* 62:1371–1414.
- Götz, F. 2004. Staphylococci in colonization and disease: prospective targets for drugs and vaccines. *Curr. Opin. Microbiol.* 7:477–487.
- Götz, F., T. Bannerman, and K. H. Schleifer. 2006. The genera *Staphylococcus* and *Micrococcus*, p. 5–75. In M. Dworkin (ed.), *The prokaryotes*, vol. 4. Springer, New York, NY.
- Graumann, P. L. 2000. *Bacillus subtilis* SMC is required for proper arrangement of the chromosome and for efficient segregation of replication termini but not for bipolar movement of newly duplicated origin regions. *J. Bacteriol.* 182:6463–6471.
- Güthlein, C., R. M. Wanner, P. Sander, E. C. Böttger, and B. Springer. 2008. A mycobacterial *smc* null mutant is proficient in DNA repair and long-term survival. *J. Bacteriol.* 190:452–456.
- Haydon, D. J., N. R. Stokes, R. Ure, G. Galbraith, J. M. Bennett, D. R. Brown, P. J. Baker, V. V. Barynin, D. W. Rice, S. E. Sedelnikova, J. R. Heal, J. M. Sheridan, S. T. Aiwale, P. K. Chauhan, A. Srivastava, A. Taneja, I. Collins, J. Errington, and L. G. Czaplewski. 2008. An inhibitor of FtsZ with potent and selective anti-staphylococcal activity. *Science* 321:1673–1675.
- Herbert, S., A. Bera, C. Nerz, D. Kraus, A. Peschel, C. Goerke, M. Meehl, A. Cheung, and F. Götz. 2007. Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. *PLoS Pathog.* 3:e102.
- Hirano, M., and T. Hirano. 2002. Hinge-mediated dimerization of SMC protein is essential for its dynamic interaction with DNA. *EMBO J.* 21:5733–5744.
- Kaimer, C., J. E. González-Pastor, and P. L. Graumann. 2009. SpoIIIE and a novel type of DNA translocase, SftA, couple chromosome segregation with cell division in *Bacillus subtilis*. *Mol. Microbiol.* 74:810–825.
- Kubitschek, H. E. 1990. Cell volume increase in *Escherichia coli* after shifts to richer media. *J. Bacteriol.* 172:94–101.
- Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu. 2001. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 357:1225–1240.
- Leibig, M., B. Krismer, M. Kolb, A. Friede, F. Götz, and R. Bertram. 2008. Marker removal in staphylococci via Cre recombinase and different *lox* sites. *Appl. Environ. Microbiol.* 74:1316–1323.
- Lindow, J. C., R. A. Britton, and A. D. Grossman. 2002. Structural maintenance of chromosomes protein of *Bacillus subtilis* affects supercoiling in vivo. *J. Bacteriol.* 184:5317–5322.
- Mascarenhas, J., J. Soppa, A. V. Strunnikov, and P. L. Graumann. 2002. Cell cycle-dependent localization of two novel prokaryotic chromosome segregation and condensation proteins in *Bacillus subtilis* that interact with SMC protein. *EMBO J.* 21:3108–3118.
- Moriya, S., E. Tsujikawa, A. K. Hassan, K. Asai, T. Kodama, and N. Ogasawara. 1998. A *Bacillus subtilis* gene-encoding protein homologous to eukaryotic SMC motor protein is necessary for chromosome partitioning. *Mol. Microbiol.* 29:179–187.
- Pilavtepe-Celik, M., M. O. Balaban, H. Alpas, and A. E. Yousef. 2008. Image analysis based quantification of bacterial volume change with high hydrostatic pressure. *J. Food Sci.* 73:M423–M429.
- Sharpe, M. E., and J. Errington. 1995. Postseptational chromosome partitioning in bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 92:8630–8634.
- She, W., Q. Wang, E. A. Mordukhova, and V. V. Rybenkov. 2007. MukEF is required for stable association of MukB with the chromosome. *J. Bacteriol.* 189:7062–7068.
- Thanbichler, M., and L. Shapiro. 2006. MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. *Cell* 126:147–162.

29. **Trevors, J. T.** 1996. Genome size in bacteria. *Antonie Van Leeuwenhoek* **69**:293–303.
30. **Volkov, A., J. Mascarenhas, C. Andrei-Selmer, H. D. Ulrich, and P. L. Graumann.** 2003. A prokaryotic condensin/cohesin-like complex can actively compact chromosomes from a single position on the nucleoid and binds to DNA as a ring-like structure. *Mol. Cell. Biol.* **23**:5638–5650.
31. **Wieland, K. P., B. Wieland, and F. Götz.** 1995. A promoter-screening plasmid and xylose-inducible, glucose-repressible expression vectors for *Staphylococcus carnosus*. *Gene* **158**:91–96.
32. **Wu, L. J., and J. Errington.** 1994. *Bacillus subtilis* SpoIIIE protein required for DNA segregation during asymmetric cell division. *Science* **264**:572–575.
33. **Yu, X. C., E. K. Weihe, and W. Margolin.** 1998. Role of the C terminus of FtsK in *Escherichia coli* chromosome segregation. *J. Bacteriol.* **180**:6424–6428.
34. **Zapun, A., T. Vernet, and M. G. Pinho.** 2008. The different shapes of cocci. *FEMS Microbiol. Rev.* **32**:345–360.