DNA-Membrane Anchor Facilitates Efficient Chromosome Translocation at a Distance in Bacillus subtilis

Nikolai P. Radzinski,a Marina Besprozvannaya,b,⁎ Eric L. McLean,b,⁎ Anusha Talwalkar,a Briana M. Burtona,b

a Department of Bacteriology, The University of Wisconsin—Madison, Madison, Wisconsin, USA
b Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, USA

ABSTRACT Chromosome segregation in sporulating Bacillus subtilis involves the tethering of sister chromosomes at opposite cell poles. RacA is known to mediate chromosome tethering by interacting with both centromere-like elements in the DNA and with DivIVA, a membrane protein which localizes to the cell poles. RacA has a secondary function in which it assists in nucleoid condensation. Here we demonstrate that, in addition to positioning and condensing the chromosome, RacA contributes to efficient transport of DNA by the chromosome segregation motor SpoIIIE. When RacA is deleted, one-quarter of cells fail to capture DNA in the nascent spore, yet 70% of cells fail to form viable spores without RacA. This discrepancy indicates that RacA possesses a role in sporulation beyond DNA capture and condensation. We observed that the mutant cells had reduced chromosome translocation into the forespore across the entire length of the chromosome, requiring nearly twice as much time to move a given DNA locus. Additionally, functional abolition of the RacA-DivIVA interaction reduced translocation to a similar degree as in a racA deletion strain, demonstrating the importance of the RacA-mediated tether in translocation and chromosome packaging during sporulation. We propose that the DNA-membrane anchor facilitates efficient translocation by SpoIIIE, not through direct protein-protein contacts but by virtue of physical effects on the chromosome that arise from anchoring DNA at a distance.

IMPORTANCE To properly segregate their chromosomes, organisms tightly regulate the organization and dynamics of their DNA. Aspects of the process by which DNA is translocated during sporulation are not yet fully understood, such as what factors indirectly influence the activity of the motor protein SpoIIIE. In this work, we have shown that a DNA-membrane tether mediated by RacA contributes to the activity of SpoIIIE. Loss of RacA nearly doubles the time of translocation, despite the physically distinct locations these proteins and their activities occupy within the cell. This is a rare example of an explicit effect that DNA-membrane connections can have on cell physiology and demonstrates that distant changes to the state of the chromosome can influence motor proteins which act upon it.

KEYWORDS Bacillus, chromosome organization, chromosome segregation, sporulation

Faithful chromosome segregation is vital for the propagation of all organisms. During mitosis, eukaryotic sister chromosomes are anchored to the spindle by kinetochores, which are multiprotein complexes and assemble specialized regions of chromosomes known as the centromere (1). The spindle apparatus uses the kinetochores to pull sister chromosomes apart toward opposite cell poles (2). While still poorly understood in general, in recent years, our understanding of similar processes occurring in bacterial cells has broadened (3). In rod-shaped bacteria, chromosome segregation is organized by the movement of the region of the chromosome containing the origin of
replication to opposite poles (4). Several recent studies have demonstrated that chromosome segregation is at least partially entropy driven and can occur spontaneously (5, 6). Yet, certain elements of chromosome segregation require highly tuned regulation. For example, regions of chromosomes that are trapped on the wrong side of the division septum undergo further segregation by RecA-like SpoIIIE/FtsK translocases (7, 8).

SpoIIIE/FtsK translocases are recruited to the division plane and transport DNA into the correct cellular compartment (7, 9). To ensure DNA is transported to the correct location, SpoIIIE/FtsK translocases recognize short noncoding sequences distributed throughout the chromosome (10–12). Most of these sequences are oriented codirectionally (~85%) and so guide the motor’s activity in the correct direction along the chromosome (9). This process of ensuring proper compartmentalization of DNA is known as “directional transport” (13).

During asymmetric division, such as occurs during sporulation in Bacillus subtilis, chromosome segregation assisted by SpoIIIE is vital for the production of a functional spore (14). FtsK/SpoIIIE is the only protein family identified as essential for chromosome segregation in all known bacteria (3–5). Upon capturing the origin-proximal 30% of the chromosome in the forespore, the SpoIIIE translocase moves 70% (3 Mb) of the forespore chromosome across the asymmetric septum during sporulation (15). During the initial stages of sporulation, both daughter chromosomes are anchored to the cell poles to ensure that DNA will be successfully captured within the forespore (16, 17). Early microscopic studies revealed that DNA capture is preceded by a change in morphology of the nucleoid from its normal diffuse shape (as observed during vegetative growth) to a compacted and extended form called the axial filament (16, 17).

RacA is a protein that binds centromere-like elements, known as ram (RacA binding motif) sites with its N-terminal helix-turn-helix motif (18, 19). These ram sites are found at high density near the origin of replication but are distributed throughout the rest of the chromosome (18). RacA contributes to chromosome condensation by binding specifically to the ram sites and nonspecifically elsewhere on the chromosome and oligomerizing, resulting in the axial filament structure and allowing for chromosome tethering at the cell poles (16–19). The oligomerization of RacA on the DNA into the axial filament is the result of both its C-terminal coiled coil and its N-terminal helix-turn-helix domains (19). The anchoring of sister chromosomes to the cell poles by RacA is the result of the interaction between the coiled coil domain of the curvature-localizing membrane-binding protein DivIVA and the RacA C-terminal coiled coil domain (16, 17, 19–22).

The primary roles established for RacA to date are to ensure a chromosome is localized for capture within a forespore and to contribute to the initiation of chromosome packaging for spore development (16, 17, 23, 24). Without RacA, ~50% of cells fail to capture a chromosome with their asymmetric septa, forming anucleate forespores (16, 17). When this occurs, a backup mechanism allows cells to put down a second asymmetric septum, thereby giving cells another chance to capture DNA (16, 17). By the end of this stage of early spore development, approximately 25% of mutant cells have failed to capture a chromosome at either cell pole (17). Additionally, capture of DNA inside the forespore is necessary for proper assembly of the SpoIIIIE motor at the septum (25). Here, we asked if RacA contributes to chromosome segregation beyond its role in packaging.

In this work, we demonstrate that a protein involved in chromosome positioning can impact the dynamics of a spatially distant DNA motor. We found that deleting RacA greatly impairs DNA translocation and that this effect is more apparent in SpoIIIIE-deficient mutants. The translocation defect of ΔracA cells becomes progressively worse along the length of the chromosome, suggesting that RacA contributes to the efficient directional movement of DNA throughout chromosome segregation and not just during the initial stages. To separate the two known functions of RacA (chromosome condensation and cell pole tethering), the tethers were abolished by disrupting the RacA-DivIVA interaction. The chromosome translocation efficiency of these cells was
impaired to a similar degree as in ΔracA cells. Together, our findings indicate that anchoring of DNA contributes to sporulation not only by localizing DNA for capture in the forespore but also via an indirect contribution to SpoIIIE translocation activity. Since the impact is not the result of direct protein-protein contacts between RacA and SpoIIIE, the effect of RacA on translocation may be due to physical changes in the chromosome that result from the polar anchor.

RESULTS

A discrepancy in sporulation values indicates an additional role of RacA. Previous studies of RacA explored its impact on chromosome positioning. These studies followed sporulating cells with 1 or 2 septa and evaluated whether DNA had been misplaced and trapped outside the forespores (17). For clarity, we will henceforth refer to DNA that is successfully localized within the forespore by a septum as being “captured.” Approximately 50% of ΔracA cells failed to capture DNA with the first septum, and this success rate continued with the placement of a second, backup septum in the cases where the first failed (17). Those data together suggest roughly one-quarter of ΔracA cells failed both attempts to capture DNA in a forespore (17). The authors of that study further reported that 50% of ΔracA cells failed to sporulate. We sought to address the 2-fold discrepancy between the fraction of cells that failed to sporulate and the smaller fraction that had failed to capture DNA, and so began by repeating the sporulation efficiency assay.

We resuspended cells in minimal medium for 24 h, and colonies were plated before and after a heat kill. Colonies were also plated at the time of resuspension in order to normalize to the presporulation cell density and thus reflect the broader effects of RacA on the sporulating cell population (16, 17). These cultures were diluted before plating so that by counting colonies (100 to 300 colonies for each condition across multiple dilutions), the fractions of cells sporulating could be calculated. We observed that 30% ± 5% of ΔracA cells successfully sporulated when normalized to the wild-type CFU (Fig. 1A). An efficiency of 31% ± 4% was similarly observed when the cells were sporulated by a different technique, sporulation by exhaustion. In this case, approximately 500 colonies were counted for each condition across multiple dilutions. Since only 25% of ΔracA cells failed to capture DNA in a forespore but 70% ± 5% failed to sporulate at all, this reexamination of DNA capture versus sporulation efficiency confirmed that RacA might have some additional function contributing to sporulation.

Because RacA is only present transiently early in sporulation, any impact on sporulation efficiency is likely to occur in this early window of time when DNA translocation is occurring (16).

RacA contributes to efficient translocation of 90° locus by SpoIIIE. To determine what other role RacA may have in sporulation, we asked if a deletion of racA affects DNA transport in vivo. To test this, we employed a previously described fluorescence assay (26). In short, a yfp reporter gene was integrated near the origin, which is always positioned in the forespore in sporulating wild-type cells. A cfp reporter gene was integrated at a region of the chromosome that is always captured in the mother cell in wild-type cells. Both reporters were placed under a promoter dependent on the forespore-specific transcription factor σ7 so that yfp and cfp were only expressed in the forespore. Thus, a yellow fluorescent protein (YFP) signal demonstrates that a septum has separated the mother cell from the forespore, within which the ori-proximal chromosome region containing yfp has been captured, while a cyan fluorescent protein (CFP) signal indicates that the region of the chromosome containing the cfp gene has been translocated from the mother cell into the forespore. This system can be used to estimate translocation rates with time-lapse microscopy by measuring the time between YFP and CFP expression in single cells or it can be used to indirectly identify changes by examining the fraction of CFP+/YFP− cells in the population over time.

In wild-type cells, the asymmetric septum initially captures 30% of the chromosome, which spans from approximately —60° (or 300°) to 40° (on a 360° circular chromosome) (27, 28). In the absence of RacA, 50% of cells do not capture DNA within the first
forespore, which causes them to try again and form a second asymmetric septum (16, 17). Those ΔracA cells which do successfully capture DNA in a forespore capture the ori region and so are properly oriented for translocation of the remaining chromosomal DNA into the forespore (9, 24, 29). Additionally, in contrast to mutant strains that lack the chromosome partitioning proteins, Soj and Spo0J, the absence of RacA does not significantly affect chromosome architecture (17, 30). To ensure we examined those cells that capture the origin in the forespore, we assayed only the cells that had YFP fluorescence in either forespore.

First, we observed the impact that a racA deletion has on translocation by following translocation of the cfp gene at the 90° locus, which is one of the first positions on the chromosome to be transported into the forespore after the formation of a division septum (27). One hundred live cells were tracked over 2 to 4 h across multiple fields of

![Graph A](image1.png)  
**A** wild type  
ΔracA  
Sporulation efficiency of wild-type and ΔracA cells. Sporulation was induced by resuspension in minimal medium for 24 h. The 30% ± 5% efficiency for ΔracA cells was calculated as the number of spores as a fraction of wild-type CFU, where normalization of cell density was conducted at resuspension. Error bars are the standard deviations between dilutions; 100 to 300 colonies were counted for each condition. An efficiency of 31% ± 4% was observed by a different technique, sporulation by exhaustion.

![Graph B](image2.png)  
**B** Distribution of translocation times measured by live-cell time-lapse imaging of YFP and CFP signals from the forespores of sporulating cells. One hundred cells were acquired for wild type (WT), SpoIIIEΔH9253 (ΔH9253), and ΔracA cells. The fraction is the proportion of the cells which saw a complete translocation event (YFP signal followed by CFP) with a given duration between the appearances of each signal. The vertical lines denote the mean translocation time in a given background, where for wild-type cells it was 11 min, SpoIIIEΔH9253 cells it was 25 min, and ΔracA cells it was 19 min. The cfp gene was at the 90° locus in each strain.

![Graph C](image3.png)  
**C** Translocation efficiency of wild-type (black) and ΔracA (gray) cells in three SpoIIIE backgrounds. DNA translocation by wild type SpoIIIE (WT), SpoIIIEΔH9253 (ΔH9253), and SpoIIIEΔγ (Δγ) of the 90° locus is shown after sporulation by resuspension; 500 to 1,000 forespores were included across 3 to 10 fields of view in technical replicates. Time points early in sporulation or with slowly sporulating strains may include 100 to 200 forespores.

**FIG 1** RacA contributes to sporulation by assisting SpoIIIE activity. (A) Sporulation efficiency of wild-type and ΔracA cells. Sporulation was induced by resuspension in minimal medium for 24 h. The 30% ± 5% efficiency for ΔracA cells was calculated as the number of spores as a fraction of wild-type CFU, where normalization of cell density was conducted at resuspension. Error bars are the standard deviations between dilutions; 100 to 300 colonies were counted for each condition. An efficiency of 31% ± 4% was observed by a different technique, sporulation by exhaustion. (B) Distribution of translocation times measured by live-cell time-lapse imaging of YFP and CFP signals from the forespores of sporulating cells. One hundred cells were acquired for wild type (WT), SpoIIIEΔH9253 (ΔH9253), and ΔracA cells. The fraction is the proportion of the cells which saw a complete translocation event (YFP signal followed by CFP) with a given duration between the appearances of each signal. The vertical lines denote the mean translocation time in a given background, where for wild-type cells it was 11 min, SpoIIIEΔH9253 cells it was 25 min, and ΔracA cells it was 19 min. The cfp gene was at the 90° locus in each strain. (C) Translocation efficiency of wild-type (black) and ΔracA (gray) cells in three SpoIIIE backgrounds. DNA translocation by wild type SpoIIIE (WT), SpoIIIEΔH9253 (ΔH9253), and SpoIIIEΔγ (Δγ) of the 90° locus is shown after sporulation by resuspension; 500 to 1,000 forespores were included across 3 to 10 fields of view in technical replicates. Time points early in sporulation or with slowly sporulating strains may include 100 to 200 forespores.
view in microfluidic chambers in order to identify the times YFP and CFP were each first detected for each individual cell (Fig. 1B). The difference between these events reflects the time of translocation from initiation of translocation until the point where the 90° locus was transported across the septum. While cells with wild-type SpoIIIE transported the 90° locus in an average of 11 min, ΔracA cells required an average of 19 min, nearly twice as much time. In comparison, the slowly translocating SpoIIIE variant SpoIIIED586A took 25 min, 2.5× longer than the wild type, recapitulating previous estimates derived from a population assay (26). It should be noted that previously, SpoIIIED586A was identified as SpoIIIED584A, with position designations based on an early genome sequence of Bacillus subtilis (26, 32, 52, 53). Updated genome sequencing has shown that the correct position for the mutant should be SpoIIIED586A and so henceforth, we will refer to it as such (31).

To screen a wider variety of conditions, the previously described assay examining the CFP/YFP ratio over time using static time points was performed. Five hundred to 1,000 forespores were counted across 3 to 10 fields of view in technical replicates. Tracking the transport of the 90° locus by wild-type SpoIIIE cells, there was still a notable difference between cells with and without RacA (Fig. 1C). As seen both in earlier work and in the doubling of translocation time in ΔracA cells with the more direct single cell assay here, even small changes detected by this assay reflect significant changes to translocation timing (26, 32). The portion of ΔracA cells that transported the CFP reporter into the forespore was consistently below that of the wild type throughout sporulation.

To better resolve differences in DNA translocation, we used two previously described variants of SpoIIIE that exhibit slower DNA translocation rates: SpoIIIEγ and SpoIIIED586A (Fig. 1C) (26, 32). SpoIIIEγ is missing the DNA interacting γ domain, which is responsible for dictating the direction of DNA transport through sensing SpoIIIE recognition sequences (SRS) within the chromosome (9, 32). The γ domain couples the recognition of SRS to the regulation of ATPase activity of the motor domain, and so deleting the γ domain results in sequence insensitivity and severely impaired ATPase activity (32, 33). Thus, SpoIIIEγ translocates DNA in vivo nearly 10-fold more slowly than wild-type SpoIIIE and exhibits a dramatic sporulation defect of over 4 orders of magnitude (9, 32). SpoIIIED586A is a variant that displays a 2.5-fold defect in the rate of DNA translocation compared to that of the wild type and exhibits a mild sporulation phenotype, producing ~80% the number of spores as the wild type (26, 34). Modeling based on the structure of a homologous protein indicates that the DS86A mutation is in the subunit interface between adjacent motor domains, which suggests that SpoIIIED586A may be impaired in the assembly of the functional hexamer (35). Interestingly, our single-cell translocation time data supports this further due to the broadening of the distribution of translocation times compared to that of the wild type, a likely outcome of stochastic disassembly of a functional complex (Fig. 1B). Thus, the mechanistic defects of SpoIIIEγ and SpoIIIED586A are likely different, and so these SpoIIIE variants were attractive candidates for studying the potential effect on translocation caused by deletion of racA.

Loss of RacA resulted in even greater impairment of translocation of the 90° locus in cells with both SpoIIIEγ and SpoIIIED586A (Fig. 1C, middle and bottom). While we observed only a 5% ± 1% reduction in cells 225 min into sporulation that successfully transported the 90° locus into the forespore with wild-type SpoIIIE when racA was deleted, ΔracA cells with SpoIIIEγ had 32% ± 1% fewer successful translocations at the same time point, a 6-fold change (Fig. 2B). Cells with SpoIIIED586A exhibited a milder defect, with 12% ± 2% fewer cells without racA transporting the 90° locus into the forespore. This was a 2-fold increase in the severity of the ΔracA defect compared to that of the wild-type SpoIIIE (Fig. 2B). As before, 500 to 1,000 forespores were counted across 3 to 10 fields of view in technical replicates. Because RacA contributes to translocation by wild-type and variant motors, the effect on transport is likely to be important for the general process of DNA movement and organization rather than one particular aspect of SpoIIIE function.
RacA contributes to efficient DNA movement of the entire chromosome. Since RacA contributes to the transport of the 90° locus, we asked whether the ΔracA DNA translocation defect extends along the length of the chromosome. To answer this question, we tracked the translocation of 117° and 138° loci, which are transported into the chromosome after the 90° locus (Fig. 2A). Again, 500 to 1,000 forespores were counted across 3 to 10 fields of view in technical replicates at each time point. The 117° and the 138° loci are ∼330 kbp and ∼590 kbp away from the 90° locus, respectively. We observed that the ΔracA defect is propagated along the length of the chromosome in SpoIIIE mutants (Fig. 2A). As with the 90° locus, wild-type SpoIIIE seems to translocate both loci with only a moderate defect with or without RacA (Fig. 2A, top). On the other hand, both SpoIIIEΔγ and SpoIIIEΔ586A translocate both the 117° and the 138° loci more rapidly in the presence of RacA than without it (Fig. 2A, middle and bottom). The impact of the ΔracA defect in transporting the 117° locus is exaggerated in comparison to the transport of the 90° locus, with 33% and 1% of SpoIIIEΔγ and SpoIIIEΔ586A cells, respectively, translocating this locus (Fig. 2A and B). The defect is further exaggerated for the 138° locus, with 47% and 27% of SpoIIIEΔγ and SpoIIIEΔ586A cells, respectively, translocating this locus (Fig. 2A and B). This progressively increasing effect demonstrates that RacA impacts DNA translocation throughout the process of chromosome segregation and not just at the chromosome capture stage (Fig. 2B).

Abolition of the chromosomal anchoring function of RacA. RacA has two previously described functions: chromosome anchoring and DNA condensation (16, 17). Since ΔracA cells exhibited a DNA translocation defect along the entire length of the chromosome, we wondered whether one or both functions of RacA contribute to efficient DNA translocation throughout the process of DNA segregation. To study this, we separated the ability of RacA to condense DNA from its ability to anchor the chromosome by deleting carboxy-terminal amino acid residues of DivIVA. Two-hybrid interaction data suggested these residues are solely responsible for interaction with RacA (16, 17, 36). DivIVA has a role in myriad cellular processes, and significant changes

![Fig 2](image-url)
to its sequence result in a growth defect, a propensity to form minicells, and extensive chaining (36). The two-hybrid data suggested deleting at least 11 amino acids from the C terminus would be sufficient to ensure abolition of the RacA-DivIVA interaction while minimizing the impact on the rest of the cell (36). In case removing 11 amino acids was not sufficient to abolish the functional intermolecular interaction, we further examined the interaction in DivIVAΔ21.

To evaluate whether the RacA-DivIVA interaction was abolished in DivIVAΔ11 and DivIVAΔ21, we quantified DNA capture in the forespore of DivIVAΔ11 and DivIVAΔ21 cells by imaging fluorescently tagged RacA-green fluorescent protein (GFP) (Fig. 3A). All data were collected 3 h after induction of sporulation by resuspension, and at least 500 forespores were counted for each strain. RacA-GFP fluorescence was not detected in 24% of wild-type or DivIVAΔ11 forespores (Fig. 3A). In contrast, 77% of divIVAΔ21 forespores failed to capture chromosomes by this assay. As expected, we saw the same defect in ΔdivIVA cells, where there should be no interaction. The discrepancy between these data and previous work indicating all wild-type cells and half of ΔracA cells fail to capture DNA is likely due to our use of a particularly stringent threshold of fluorescence detection (16). To get a more precise and independent measure of the RacA-DivIVA interaction, we took an approach to more directly visualize the tether in a system with wild-type RacA.

FIG 3 Deletion of 21 residues from the C terminus of DivIVA abolishes its interaction with RacA. (A) Sample images of RacA-GFP in wild-type cells stained with FM4-64. Bar, 1 μm. White arrowheads point to forespores empty of DNA (left) and containing DNA (right). (B) Fractions of forespores with (gray) and without (black) detectable RacA-GFP fluorescence 180 min after sporulation by resuspension. At least 500 forespores were counted for each strain. (C) Sample images of TetR-mCherry in wild-type cells stained with TMA-DPH and used for line scans. Bar, 1 μm. The white arrowhead denotes a TetR-mCherry focus. (D) Diagram of line scan (black line) through a forespore, intersecting a TetR-mCherry focus (black circle) and the closest region of TMA-DPH-stained membrane. These line scans were used for calculating the distance d between the locus and the membrane. (E) Distributions of distances calculated between each focus and the nearest portion of the membrane. For wild-type cells, the mean distance was 83 nm (upper black vertical line) and for DivIVAΔ11 cells, the mean was 107 nm, while for DivIVAΔ21 cells, the mean was 162 nm (lower black vertical line). One hundred line scans were performed for each strain.
To further verify the RacA-DivIVA interactions were abolished in DivIVAΔ21 cells, we directly visualized the intracellular position of a specific ori-proximal locus with respect to the cell membrane (Fig. 3B and C). We inserted an array of Tet repressor binding sites (tetO) into the chromosome at the yycR locus where the ram sites targeted by RacA are at a high density. TetR-mCherry was expressed under a xylose-inducible promoter, and distinct foci could be visualized. One hundred foci were evaluated for each strain. The position of a given TetR-mCherry focus was determined by aligning a line scan with the closest portion of TMA-DPH [1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluene sulfonate]-stained membrane and fitting a window of pixels around the peak intensity to a Gaussian distribution (Fig. 3B). A Gaussian fit of the pixels around the peak TMA-DPH intensity on the line scan provided the membrane position so that the distance between the focus and the cell membrane could be measured.

We observed that wild-type cells had a mean distance of 83 nm between foci and the membrane (Fig. 3C, top). The distribution of distances was similar in DivIVAΔ11 cells, with a mean distance of 107 nm (middle). Cells containing divIVAΔ21, however, had a mean distance of 162 nm between foci and the membrane (bottom). The near doubling in mean distance of the focus from the membrane between wild-type and DivIVAΔ21 cells suggests that the RacA-DivIVA interaction is functionally abolished in DivIVAΔ21 cells. While RacA-GFP localization and TetR-mCherry localization are indirect measurements of the DivIVA-RacA interaction, taken together with the earlier two-hybrid data, they strongly indicate that the functional interaction has been effectively abolished in DivIVAΔ21 to the point where the tether itself can be satisfactorily described as no longer present.

**Chromosome anchoring versus condensation.** Having established that anchoring is functionally abolished in DivIVAΔ21 cells where RacA remains intact, we could now specifically explore the effect of chromosome anchoring on DNA translocation. We examined DivIVAΔ21 cells with the YFP-CFP assay described previously, again evaluating 500 to 1,000 forespores at each time point (Fig. 4A and B). As described above, cells contained either wild-type SpoIIIE (top), SpoIIIE<sup>D586A</sup> (middle), or SpoIIIE<sup>Δy</sup> (bottom).
When cfp was located at the 90° locus, translocation efficiency reduced by 5% ± 2% in wild-type SpoIIIE cells with DivIVAΔ21 180 min into sporulation. This is close to the 7% ± 1% reduction observed in ΔracA cells. Translocation efficiency was indistinguishable between wild-type and DivIVAΔ21 cells. The impact of the defect in DivIVAΔ21 cells and ΔracA cells was likewise similar in SpoIIIEΔγ and SpoIIIEΔ586A backgrounds. Taken together, these data indicate anchoring of DNA to the membrane by RacA contributes to SpoIIIE activity and that any contribution of DNA compaction is negligible in comparison.

**DISCUSSION**

The data presented here indicate that the chromosome tethering activity of RacA contributes to DNA translocation by SpoIIIE. We show that RacA has an additional role beyond capturing DNA in the nascent forespore and condensing the chromosome. The tether anchors DNA to the membrane and provides a long-range contribution to efficient chromosome segregation.

The translocation defect for wild-type SpoIIIE in a ΔracA background became more apparent in the context of variants, SpoIIIEΔγ and SpoIIIEΔ586A. This apparently milder impact of ΔracA on wild-type SpoIIIE activity is likely because the wild-type motor translocates DNA with a velocity near the limit of time resolution of the \textit{in vivo} fluorescence DNA transport assay employed here. In fact, small deficiencies identified with this assay actually indicate significant reductions in translocation time, as seen in the live-cell quantification of translocation time, where ΔracA nearly doubled the time it took to transport DNA (Fig. 1B). The effects on the activity of the motor observed here in assays of translocation are significant not just by their magnitude but further by the nature of the impact. The tether is spatially separated by as much as hundreds of nanometers from the motor complex and yet assists the motor throughout chromosome translocation.

Interestingly, the impacts of SpoIIIEΔ586A and ΔracA on sporulation were relatively similar. SpoIIIEΔ586A took 2.5-fold longer than SpoIIIE to complete translocation and had a 6% reduction in CFP/YFP at the 90° locus 180 min after initiation of sporulation, while ΔracA cells took 2-fold longer and had a 7% reduction in CFP/YFP at the same time point (Fig. 1B and C). SpoIIIEΔ586A cells had a sporulation efficiency defect of ∼80% that of the wild type, most likely stemming from this reduction in translocation rate (26, 34). The discrepancy between the measured sporulation efficiency and the rate of capture of DNA in ΔracA cells which initially inspired this work may in fact be explained by this similar reduction in translocation rate. The timing of sporulation, particularly at the time of translocation, is highly well regulated. Movement of sporulation regulators along the length of the chromosome can reduce sporulation efficiency by up to an order of magnitude (26, 37, 38). Yet in these cases, the change in timing for the regulator to move into the forespore increases by, at most, one-third. In contrast, the racA deletion doubles translocation time.

There have been numerous previous cases where even relatively small effects on sporulation efficiency can actually reflect significant roles in sporulation. For example, a recent transposon screen for \textit{Bacillus subtilis} sporulation factors still missed 15 of 148 known genes with roles in sporulation, despite identifying several new factors (39). Several early screens even missed RacA entirely, despite its roles in chromosome capture, SpoIIIE complex nucleation, and efficient chromosome transport (16, 25, 40–42).

A number of forespores in ΔracA cells expressed CFP, but not YFP, and these were excluded from the YFP/CFP fraction calculations, as the lack of YFP suggested that the chromosomes had been improperly captured in the forespore. We found that ∼10% of forespores expressing CFP did not also have a detectable YFP signal in wild-type SpoIIIE cells with the cfp gene located at the 90° locus (see Table S1 in the supplemental material). This number aligns with results from a previous study in which fluorescently labeled chromosomal loci were localized in order to identify which regions of the chromosome had been captured in the forespore in a nontranslocating SpoIIIE back-
ground (27, 29). Those results found that ~10% of ΔracA cells which successfully captured DNA in the forespore had failed to capture ori (29). We saw that the further from ori the cfp gene was located, the less often it was expressed without an accompanying YFP signal. This is likely because the greater distance makes it more improbable for the region to be mistakenly captured in the forespore (Table S1). Interestingly, the CFP-only fraction also decreased as the severity of the SpoIIIE defect increased, such that cells with wild-type SpoIIIE were the most likely to mistakenly express CFP alone. It is unclear why this population decreases, but it could be related to the specific mechanistic defects of SpoIIIE<sup>ΔD586A</sup> and SpoIIIE<sup>Δy</sup> as well as the organization of the chromosome at the septum.

After the RacA-DivIVA interaction was abolished by deleting 21 residues from the C-terminal end of DivIVA, we observed a significant reduction in the number of forespores successfully capturing chromosomes compared to that for cells with wild-type DivIVA (Fig. 3A). We provided more direct evidence for the abolition of tethering in mutant divIVA cells by fluorescently labeling an origin-proximal locus. The distance between the fluorescent focus and the membrane in forespores increased from 83 nm in the wild type to 162 nm in divIVAΔ21 cells (Fig. 3C). The greater distance between DNA and membrane indicates it is more likely the DNA is not directly attached to the cell poles and is instead free to diffuse elsewhere in the forespore. Because the microscope images are merely two-dimensional (2D) projections of a three-dimensional (3D) structure, any information regarding distances along the z axis is lost, and the values measured are not absolute but relative distances. Additionally, assuming a 600-nm-diameter forespore, a freely diffusing particle will appear an average of 174 nm away from the membrane in a one-dimensional (1D) line scan. Although previous studies proposed that a Soj-mediated DNA-membrane interaction could exist in sporulating cells, the small difference between the measured average distance of 162 nm and the predicted 174 nm could indicate that, even in the absence of RacA, these Soj-mediated interactions do not play a significant role (29).

DivIVAΔ11 cells surprisingly did not show mislocalization of RacA-GFP despite the indication from the earlier two-hybrid experiments that this may be sufficient to abolish the interaction with RacA (Fig. 3B) (36). However, the localization of the TetR-mCherry focus in DivIVAΔ11 cells to an average of 107 nm from the membrane is slightly greater than the 83 nm measured in wild-type cells, indicating that, while the tether may be somewhat impaired, it is still at least transiently present (Fig. 3C). Because the RacA-GFP localization indicates DNA is still captured as successfully as in wild-type cells and the translocation efficiency of DivIVAΔ11 cells is indistinguishable from that of wild-type cells, the tethers have not been actually functionally abolished (Fig. 4A). The discrepancy between the data indicating there is some abolition of activity (the two-hybrid assay and the distance between a DNA locus and the membrane) and the data indicating there is no change (the RacA-GFP localization and YFP/CFP assays) suggests that even a reduced interaction is sufficient for maintaining the tether.

While we now understand that DNA-membrane tethers indirectly contribute to SpoIIIE activity, the precise mechanism of this effect remains unclear. There are only a few other examples of DNA-membrane interactions in bacteria to compare to (43). DNA-membrane tethers have been hypothesized to change the general architecture of the chromosome by providing an expansion force to contrast the myriad compacting forces which condense the volume of the nucleoid (44). This expansion force is believed to provide macromolecules the ability to freely interact with the chromosome (43). The ΔracA defect we observed persisted regardless of the SpoIIIE background and throughout the process of translocation. This combined with the distance between the tether and the SpoIIIE complex and the lack of known partners that could facilitate a direct interaction between RacA and SpoIIIE suggests that the tether produces a global physical effect. One possibility that will be a subject of future studies is that the tether may reorganize the chromosome itself.

A possible explanation for how a tether could influence translocation is by providing an expansion force for the chromosome. Because of the high rate at which SpoIIIE
translocates, it may be important for the DNA polymer to remain mobile in order to be
drawn toward the SpoIIIE complex as quickly as it is pumped through it. By sequest-
ering the ori-proximal region of the chromosome to the pole of the forespore, the
chromosome should more efficiently fill the volume available, as the tether would
provide a counter to the many compacting forces that reduce DNA mobility (43, 44).
Proper packaging of DNA into the forespore is important, particularly as the forespore
swells with DNA through sporulation (45). However, SpoIIIE has no problem stripping
off large quantities of DNA-bound protein at a high rate during translocation, and so
it is not yet obvious how sensitive the motor is to local or global DNA structure
(34). Additionally, the other role of RacA in compacting the chromosome appears to
contradict the idea that it would provide an expansion force. Regardless of the specific
mechanism, the effects of DNA-membrane tethering by RacA on the chromosome are
likely to be large scale and suggest that even a strong motor such as SpoIIIE may be
influenced by long-range interactions and the global state of cellular DNA.

MATERIALS AND METHODS

Bacterial growth conditions. All B. subtilis strains are given in Table S2 and all primers are given in
Table S3 in the supplemental material. Strains were derived from laboratory prototrophic strain PY79 (46).
Transformation of B. subtilis was performed with double-stranded PCR fragments, B. subtilis genomic
DNA, or linearized plasmid (47). Synchronized sporulation was induced by resuspension in minimal
medium at A600 of 0.6 at 37°C (48). All plasmids were propagated in Escherichia coli strain DH5α, which
was grown and transformed as previously described (49). TetR-mCherry-containing strains were main-
tained in 40 ng/ml anhydrotetracycline (aTc) in order to inhibit TetR-mCherry binding to tetO sites. Additionally, 6% (wt/vol) xylose was added to the medium 60 min before resuspension into minimal
medium to induce expression of TetR-mCherry. Xylose and aTc were each not included in minimal
medium after resuspension. Cell membranes were stained with either 50 µM TMA-DPH or 2.5 µM FM4-64
in 1× phosphate-buffered saline (PBS). Sporulation efficiency assays were performed as previously
described (47).

Cloning. For two-color DNA transport assay, long-flanking PCR was performed to insert Pori-cfp (tet) into yhodG (90°), ykcC (117°), and yfA (138°). All clones were propagated in E. coli DH5α cells and verified
by sequencing. Oligonucleotides and strain information are provided in the supplemental material.

Image analysis. Image analysis was performed with FIJI (Fiji Is Just ImageJ) (50). CFP and YFP experiment quantification was performed manually. During identification of both YFP+ and CFP+
forespores, the minimum display range was set as low as possible so as to include any detectable signal,
and any forespore twice as bright as the noise above background fluorescence was counted. For live-cell
time-lapse experiments, the first time point YFP or CFP was detected was noted for each cell, and the
difference between the two values was calculated. For bulk experiments with static time points,
forespores with YFP expression were identified and counted. Then, forespores with YFP and CFP
expression were identified and counted. Five hundred to 1,000 forespores were included for most time
points, with multiple fields of view included for each sample. Some early time points and strains with
sporulation defects had smaller numbers (n = 100 to 200).

For TetR-mCherry focus colocalization with the membrane, cells which contained a forespore visible
with the TMA-DPH stain were identified after a single iteration of the FIJI smoothing function was
applied. If the forespore had exactly one TetR-mCherry focus, a 2D line scan was made between the focus
and the closest region of the membrane. The position of the focus was identified by fitting the peak
intensity pixel and a 7-pixel-wide window around it to a Gaussian distribution where y = A e^{-(x-a)^2/2σ^2} and
a is the center of the peak. The closest TMA-DPH fluorescence intensity peak from here on the line scan
was then identified and similarly fit to a Gaussian distribution. The distance was calculated between these
two subpixel positions. One hundred line scans were performed for each experiment, collected across
several individual fields of view. Images included in figures underwent a single iteration of the FIJI
smoothing function.

Fluorescence microscopy. Fluorescence microscopy was performed on an inverted Zeiss Ob-
server.Z1 and an upright Zeiss Imager.M1, each with a Photometrics CoolSNAP HQ2 camera. The data were
collected on the upright microscope as previously described (26). Samples stained with TMA-DPH
were imaged on the inverted microscope (this includes some images of the CFP/YFP translocation assay
in containing divIVA variants in Fig. 4 as well as all images of the TetR-mCherry foci in Fig. 3). Fluorescence
was activated with a Zeiss Colibri light-emitting diode (LED) light source. TMA-DPH was activated with
a 353-nm LED for 1 s at 100% source intensity. TetR-mCherry was activated with a 589-nm LED for 2 to
10 s at 100% source intensity. Images were acquired with the Zeiss ZEN 2.3 software package. All
white-light images were obtained using phase-contrast microscopy.

For time-lapse imaging, images were acquired every 2 min with tilting, define focus, and a constant
flow of medium through a fabricated polydimethylsiloxane (PDMS) microfluidic chamber. Depleted
medium was prepared by sporulating wild-type PY79 cells by resuspension, spinning down the culture
2.5 h into sporulation, and filtering the supernatant in a 0.2-µm filter. Cells were adhered to the glass
surface of the microfluidic device by incubating for 10 to 20 min with 0.03% (wt/vol) chitosan, which had
been freshly dissolved in 0.1 M acetic acid several hours before addition to the device. The chambers
were then rinsed with water and depleted sporulation medium before cells were added (S1). Heavy flow was applied to flatten cells onto the surface, and a constant flow of depleted medium maintained sporulation conditions. Time-lapse images were taken over 2 to 4 h, where every 2 min, phase-contrast, YFP, and CFP snapshots were taken. Phase contrast used a 20-ms exposure with a 6.4 V TL halogen lamp, YFP was activated with a 505-nm LED at 10% intensity for 1 s, and CFP was activated with a 445-nm LED at 10% intensity for 1 s.

SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at https://doi.org/10.1128/mBio.01117-19.

TABLE S1, DOCX file, 0.1 MB.
TABLE S2, DOCX file, 0.1 MB.
TABLE S3, DOCX file, 0.1 MB.

ACKNOWLEDGMENTS
We thank David Z. Rudner for extensive discussions and feedback during the project, Dong K. Shin for help with quantitative analysis, Mark Jedras for assistance with strain construction, and Katrina Forest for critical reading of the manuscript.

This work was supported by National Institutes of Health (NIH) (GM121865) and Massachusetts Life Science Foundation Grant (to B.M.B.). M.B. was supported by a National Science Foundation Graduate Research Fellowship (DGE-1144152) and by the Molecules and Organisms NIH Training Grant (GM07598). E.L.M. was supported by an NIH R25 grant (GM109436) awarded to Sheila M. Thomas.

N.P.R., M.B., and B.M.B. designed the research. N.P.R., M.B., A.T., and E.L.M. performed the research. N.P.R., M.B., A.T., E.L.M., and B.M.B. contributed new reagents/analytic tools. N.P.R., M.B., and E.L.M. analyzed the data. N.P.R., M.B., and B.M.B. wrote the paper.

REFERENCES
1. Yanagida M. 2005. Basic mechanism of eukaryotic chromosome segregation. Philos Trans R Soc Lond B Biol Sci 360:609–621. https://doi.org/10.1098/rstb.2004.1615.
2. Rago F, Cheeseman IM. 2013. The functions and consequences of force at kinetochores. J Cell Biol 200:557–565. https://doi.org/10.1083/jcb_201211113.
3. Badrinarayanan A, Le TBK, Laub MT. 2015. Bacterial chromosome organization and segregation. Annu Rev Cell Dev Biol 31:171–199. https://doi.org/10.1146/annurev-cellbio-100814-125211.
4. Wang X, Llopis PM, Rudner DZ. 2013. Organization and segregation of bacterial chromosomes. Nat Rev Genet 14:191–203. https://doi.org/10.1038/nrg3375.
5. Youngren B, Nielsen HJ, Jun S, Austin S. 2014. The multifork Escherichia coli chromosome is a self-duplicating and self-segregating thermodynamic ring polymer. Genes Dev 28:71–84. https://doi.org/10.1101/gad.231050.113.
6. Jun S, Mulder B. 2006. Entropy-driven spatial organization of highly confined polymers: lessons for the bacterial chromosome. Proc Natl Acad Sci U S A 103:12388–12393. https://doi.org/10.1073/pnas.0605305103.
7. Crozat E, Grainge I. 2010. FtsK DNA translocase: the fast motor that knows where it’s going. Chembiochem 11:2232–2243. https://doi.org/10.1002/cbic.201000347.
8. Kaimer C, Graumann PL. 2011. Players between the worlds: multifunctional DNA translocases. Curr Opin Microbiol 14:719–725. https://doi.org/10.1016/j.mib.2011.09.004.
9. Pracan JL, Nollmann M, Becker EC, Cozzarelli NR, Pogliano K, Bustamante C. 2008. Sequence-directed DNA export guides chromosome translocation during sporulation in Bacillus subtilis. Nat Struct Mol Biol 15:485–493. https://doi.org/10.1038/nsmb.1412.
10. Bigot S, Saleh OA, Cornet F, Allemand J-F, Barre F. 2006. Oriented loading of FtsK on KOPS. Nat Struct Mol Biol 13:1026–1028. https://doi.org/10.1038/nsmb1159.
11. Pracan JL, Nollmann M, Bustamante C, Cozzarelli NR. 2006. Identification of the FtsK sequence-recognition domain. Nat Struct Mol Biol 13:1023–1025. https://doi.org/10.1038/nsmb1157.
12. Sivanathan V, Allen MD, de Bekker C, Baker R, Arsiczewska LK, Freund SM, Bycroft M, Löwe J, Sherratt DJ. 2006. The FtsK γ domain directs oriented DNA translocation by interacting with KOPS. Nat Struct Mol Biol 13:965–972. https://doi.org/10.1038/nsmb1158.
13. Besprozvannaya A, Burton BM. 2014. Do the same traffic rules apply? Directional chromosome segregation by SpoIIIE and FtsK. Mol Microbiol 93:599–608. https://doi.org/10.1111/mmi.12708.
14. Piggot PJ. 1973. Mapping of asporogenous mutations of Bacillus subtilis: a minimum estimate of the number of sporulation operons. J Bacteriol 114:1241–1253.
15. Errington J, Bath J, Wu LJ. 2001. DNA transport in bacteria. Nat Rev Mol Cell Biol 2:538–545. https://doi.org/10.1038/35080005.
16. Ben-Yehuda S, Rudner DZ, Losick R. 2003. RacA, a bacterial protein that anchors chromosomes to the cell poles. Science 299:532–536. https://doi.org/10.1126/science.1079914.
17. Wu LJ, Errington J. 2003. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating Bacillus subtilis. Mol Microbiol 49:1463–1475. https://doi.org/10.1046/j.1365-2958.2003.03643.x.
18. Ben-Yehuda S, Fujita M, Liu XS, Gorbatyuk B, Skoko D, Yan J, Marko JF, Liu JS, Eichenberger P, Rudner DZ, Losick R. 2005. Defining a centromere-like element in Bacillus subtilis by identifying the binding sites for the chromosome-anchoring protein RacA. Mol Cell 17:773–812. https://doi.org/10.1016/j.molcel.2005.02.023.
19. Schumacher MA, Lee J, Zeng W. 2016. Molecular insights into DNA binding and anchoring by the Bacillus subtilis sporulation kinetochore-like RacA protein. Nucleic Acids Res 44:5438–5449. https://doi.org/10.1093/nar/gkw248.
20. Lenarcic R, Hallbedel S, Visser L, Shaw M, Wu LJ, Errington J, Marenduzzo D, Hamon LW. 2009. Localisation of DivIVA by targeting to negatively curved membranes. EMBO J 28:2227–2228. https://doi.org/10.1038/emboj.2009.129.
21. Ramamurthi KS, Losick R. 2009. Negative membrane curvature as a cue for subcellular localization of a bacterial protein. Proc Natl Acad Sci U S A 106:13541–13545. https://doi.org/10.1073/pnas.0906851106.
22. Thomaides HB, Freeman M, El KM, Errington J. 2001. Division site selection protein DivIVA of Bacillus subtilis has a second distinct function in chromosome segregation during sporulation. Genes Dev 15:1662–1673. https://doi.org/10.1101/gad.197501.
23. Burton B, Dubnau D. 2010. Membrane-associated DNA transport ma-

May/June 2019 Volume 10 Issue 3 e01117-19
mbio.asm.org 12
24. Becker EC, Pogliano K. 2007. Cell-specific SpoIIIE assembly and DNA translocation polarity are dictated by chromosome orientation. Mol Microbiol 66:1066–1079. https://doi.org/10.1111/j.1365-2958.2007.05992.x.

25. Ben-Yehuda S, Rudner DZ, Losick R. 2003. Assembly of the SpoIIIE DNA translocase depends on chromosome trapping in Bacillus subtilis. Curr Biol 13:2196–2200. https://doi.org/10.1016/j.cub.2003.12.001.

26. Burton BM, Marquis KA, Sullivan NL, Rapoport TA, Rudner DZ. 2007. The ATPase SpoIIIE transports DNA across fused septal membranes during sporulation in Bacillus subtilis. Cell 131:1301–1312. https://doi.org/10.1016/j.cell.2007.04.044.

27. Sullivan NL, Marquis KA, Rudner DZ. 2009. Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. Cell 137:679–707. https://doi.org/10.1016/j.cell.2009.04.046.

28. Wu LJ, Errington J. 1994. Bacillus subtilis SpoIIIE protein required for DNA segregation during asymmetric cell division. Science 264:572–575. https://doi.org/10.1126/science.8160014.

29. Kloosterman TG, Lenarcic R, Willis CR, Roberts DM, Hamoen LW, Errington J, Wu LJ. 2016. Complex polymer machinery required for proper chromosome segregation in vegetative and sporulating cells of Bacillus subtilis. Mol Microbiol 101:333–350. https://doi.org/10.1111/mmi.13393.

30. Sharpe ME, Errington J. 1996. The Bacillus subtilis soj-spooJ locus is required for a centromere-like function involved in prespore chromosome partitioning. Mol Microbiol 21:501–509. https://doi.org/10.1111/j.1365-2958.1996.tb02599.x.

31. Zhu B, Stulke J. 2018. SubtiWiki in 2018: from genes and proteins to functional network annotation of the model organism Bacillus subtilis. Nucleic Acids Res 46:D743–D748. https://doi.org/10.1093/nar/gkx908.

32. Besprozvannaya M, Pivorunas VL, Feldman Z, Burton BM. 2013. SpoIIE protein achieves directional DNA translocation through allosteric regulation of ATPase activity by an accessory domain. J Biol Chem 288:28962–28974. https://doi.org/10.1074/jbc.M113.484055.

33. Cattoni DI, Chara O, Godsfrey C, Marguet E, Trigueros S, Milhiet P-E, Nollmann M. 2013. SpoIIE mechanism of directional translocation involves target search coupled to sequence-dependent motor stimulation. EMBO Rep 14:473–479. https://doi.org/10.1038/embor.2013.39.

34. Marquis KA, Burton BM, Nollmann M, Ptacin JL, Bustamante C, Ben-Yehuda S, Rudner DZ. 2008. SpoIIIE strips proteins off the DNA during chromosome translocation. Genes Dev 22:1786–1795. https://doi.org/10.1101/gad.1684008.

35. Bose B, Reed SE, Besprozvannaya M, Burton BM. 2016. Missense mutations allow a sequence-blind mutant of SpoIIIE to successfully translocate chromosomes during sporulation. PLoS One 11:e0148366. https://doi.org/10.1371/journal.pone.0148366.

36. van Baarle S, Celik IN, Kaval KG, Bramkamp M, Hamoen LW, Halbedel S. 2013. Protein–protein interaction domains of the soj-spooJ locus are required for DNA translocation in Bacillus subtilis. J Bacteriol 195:1012–1021. https://doi.org/10.1128/JB.02171-12.

37. Frandsen N, Barak I, Karmayzam-Campelli C, Stragier P. 1999. Transient asymmetric geometry during sporulation and establishment of cell specificity in Bacillus subtilis. Genes Dev 13:394–399. https://doi.org/10.1101/gad.13.4.394.

38. Khvorova A, Chary VK, Hilbert DW, Piggot PJ. 2000. The chromosomal location of the Bacillus subtilis sporulation gene spoIIIR is important for its function. J Bacteriol 182:4425–4429. https://doi.org/10.1128/JB.182.16.4425-4429.2000.

39. Meeske AJ, Rodrigues CDA, Brady J, Lim HC, Bernhardt TG, Rudner DZ. 2016. High-throughput genetic screens identify a large and diverse collection of new sporulation genes in Bacillus subtilis. PLoS Biol 14: e1002341. https://doi.org/10.1371/journal.pbio.1002341.

40. Freetz E, Fortnagel P. 1967. Analysis of sporulation mutants. I. Response of uracil incorporation to carbon sources, and other mutant properties. J Bacteriol 94:1957–1969.

41. Takahashi I. 1965. Transduction of sporogenesis in Bacillus subtilis. J Bacteriol 89:294–298.

42. Bogerov AY, Halbedel S. 1968. Genetic mapping of a locus which regulates the production of pigment associated with spores of Bacillus subtilis. J Bacteriol 95:2426–2427.

43. Roggiani M, Goulain M. 2015. Chromosome-membrane interactions in bacteria. Annu Rev Genet 49:115–129. https://doi.org/10.1146/annurev-genet-112114-054958.

44. Baski S, Choi H, Mondal J, Weisshaar JC. 2014. Time-dependent effects of transcription- and translation-halting drugs on the spatial distributions of the Escherichia coli chromosome and ribosomes. Mol Microbiol 89:871–887. https://doi.org/10.1111/mmi.12805.

45. Lopez-Garrido J, Ojkic N, Kanna H, Wagner FR, Villa E, Endres RG, Pogliano K. 2018. Chromosome translocation inflates Bacillus forespores and impacts cellular morphology. Cell 172:758. https://doi.org/10.1016/j.cell.2018.01.027.

46. Youngman PJ, Perkins JB, Losick R. 1983. Genetic transposition and insertional mutagenesis in Bacillus subtilis with Streptococcus faecalis transposon Tn197. Proc Natl Acad Sci U S A 80:2305–2309. https://doi.org/10.1073/pnas.80.8.2305.

47. Wilson GA, Bott FK. 1968. Nutritional factors influencing the development of competence in the Bacillus subtilis transformation system. J Bacteriol 95:1439–1449.

48. Harwood CR, Cutting SM. 1990. Molecular biological methods for Bacillus subtilis. ASM Press, Washington, DC.

49. Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

50. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Ruden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682. https://doi.org/10.1038/nmeth.2019.

51. Cattoni DI, Fiche J-B, Valeri A, Mignot T, Nollmann M. 2013. Super-resolution imaging of bacteria in a microfluidics device. PLoS One 8:e76268. https://doi.org/10.1371/journal.pone.0076268.

52. Moszer I, Jones LM, Moreira S, Fabry C, Danchin A. 2002. SubtiList: the reference database for the Bacillus subtilis genome. Nucleic Acids Res 30:62–65. https://doi.org/10.1093/nar/30.1.62.

53. Kunst F, Gogaswara N, Moszer I, Albertini AM, Alloni G, Azevedo V, Bertero MG, Besiéres P, Bolotin A, Borchert S, Borsis R, Boursier L, Brans A, Braun M, Brignell SC, Bron S, Brouillet S, Bruschi CV, Caldwell B, Capuano V, Carter NM, Choi SK, Codani J-J, Connerton IF, Cummings NJ, Daniel RA, Denizot F, Devine KM, Dusterhoff A, Ehrlich SD, Emmerson PT, Entian KD, Errington J, Fabret S, Ferrari E, Foulger D, Fritz C, Fujita M, Fujita Y, Fuma S, Galizzii A, Galleron N, Ghmin S-Y, Glaser P, Goffeau A, Golightly EJ, Grandi G, Guiseppi G, Guy BJ, Haga K, et al. 1997. The complete genome sequence of the Gram-positive bacterium Bacillus subtilis. Nature 390:249–256. https://doi.org/10.1038/36786.