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Assembly of the SpoIIIE DNA Translocase Depends on Chromosome Trapping in *Bacillus subtilis*

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Summary

Sporulation in *Bacillus subtilis* is an attractive system in which to study the translocation of a chromosome across a membrane. Sporulating cells contain two sister chromosomes that are condensed in an elongated axial filament with the origins of replication anchored at opposite poles of the sporangium [1, 2]. The subsequent formation of a septum near one pole divides the sporangium unequally into a forespore (the smaller compartment) and a mother cell [3]. The septum forms around the filament, trapping the origin-proximal region of one chromosome in the forespore. As a consequence, the trapped chromosome transverses the septum with the remainder being left in the mother cell [4]. Next, SpoIIIE assembles at the middle of the septum to create a translocase that pumps the origin-distal, two-thirds of the chromosome into the forespore [5]. Here, we address the question of how the DNA translocase assembles and how it localizes to the septal midpoint. We present evidence that DNA transversing the septum is an anchor that nucleates the formation of the DNA translocase. We propose that DNA anchoring is responsible for the assembly of other SpoIIIE-like DNA translocases, such as those that remove trapped chromosomes from the division septum of cells undergoing binary fission.

Results and Discussion

Previous work has shown that formation of the axial filament and the attachment of the origin regions to opposite poles of the sporangium are mediated by a developmental protein called RacA that is produced at the start of sporulation [6]. Mutant sporangia lacking RacA do not form an axial filament. Instead, they contain a stubby nucleoid whose ends are not attached to the cell poles. As a consequence, asymmetric division frequently results in forespores that are devoid of DNA. However, polar anchoring of the chromosome and axial filament formation are not essential for chromosomal trapping, as the polar septum is frequently (about 50% of polar divisions) able to trap DNA in mutant sporangia that lack RacA. In such cases, a chromosome can be successfully translocated into the forespore. Even in cases in which the polar septum fails to trap DNA, spore formation can often still take place by the following fail-safe mechanism. Both poles of the sporangium are potential division sites; if the first-formed polar septum fails to capture DNA, a second septum forms at the distal pole, providing a second opportunity to trap DNA [6].

This ability of a RacA mutant both to produce polar septa that either have or have not trapped a chromosome provided an attractive opportunity to address the question of whether assembly of the SpoIIIE DNA translocase at the middle of the septum is dependent upon DNA that transverses the septum. SpoIIIE is a member of a family of DNA translocases that are involved in pumping DNA across membranes. SpoIIIE and its relatives are composed of an N-terminal, integral membrane domain and a C-terminal cytoplasmic domain, which contains an ATP binding site [7, 8]. The N-terminal part of SpoIIIE is required for proper localization of the protein to the division septum [5]. The C-terminal domain contains a DNA-dependent adenosine triphosphatase (ATPase) and is capable of tracking along DNA in the presence of ATP. It is this ATP-dependent tracking activity that is presumably responsible for the ability of the membrane-anchored SpoIIIE to translocate DNA [9].

The assembly of SpoIIIE in the septum can be readily visualized using a fusion of the DNA translocation protein to the green fluorescence protein (GFP). Cells producing such a SpoIIIE-GFP fusion exhibit a distinct focus of fluorescence approximately at the midpoint of the polar septum [5, 10], and it was proposed that this focus represents a channel around the trapped chromosome where it transverses the septum [5]. To carry out our analysis, we replaced *spoIIIE* with a *spoIIIE-gfp* construct in wild-type cells and in a *racA* mutant to create strains SB234 and SB237, respectively. The SpoIIIE-GFP fusion was fully, or at least substantially, functional, as cells producing the fusion protein were able to grow and sporulate as well as cells producing the unmodified DNA translocase. Developing cells of the two strains were treated with the vital membrane stain FM4-64 (red) and the DNA stain DAPI (blue) and visualized by fluorescence microscopy at various times after the start of sporulation. A typical pattern of SpoIIIE-GFP localization for the wild-type sporangia is presented in Figure 1A and in the field of cells in Figure 1F, in which the fusion protein can be seen as a bright green focus near the midpoint of the polar septa. A comparison of Figures 1F and 1G shows that septal foci were more frequently observed in wild-type sporangia than in mutant sporangia. Importantly, the presence of a focus in the polar septa of RacA mutant sporangia was invariably associated with the presence of a chromosome crossing that septum (compare Figures 1A and 1F with Figures 1B and 1G).

As indicated above, RacA mutant sporangia that fail to capture DNA following an initial round of polar division frequently form a second septum at the distal pole. The second polar division enables the sporangia to get a

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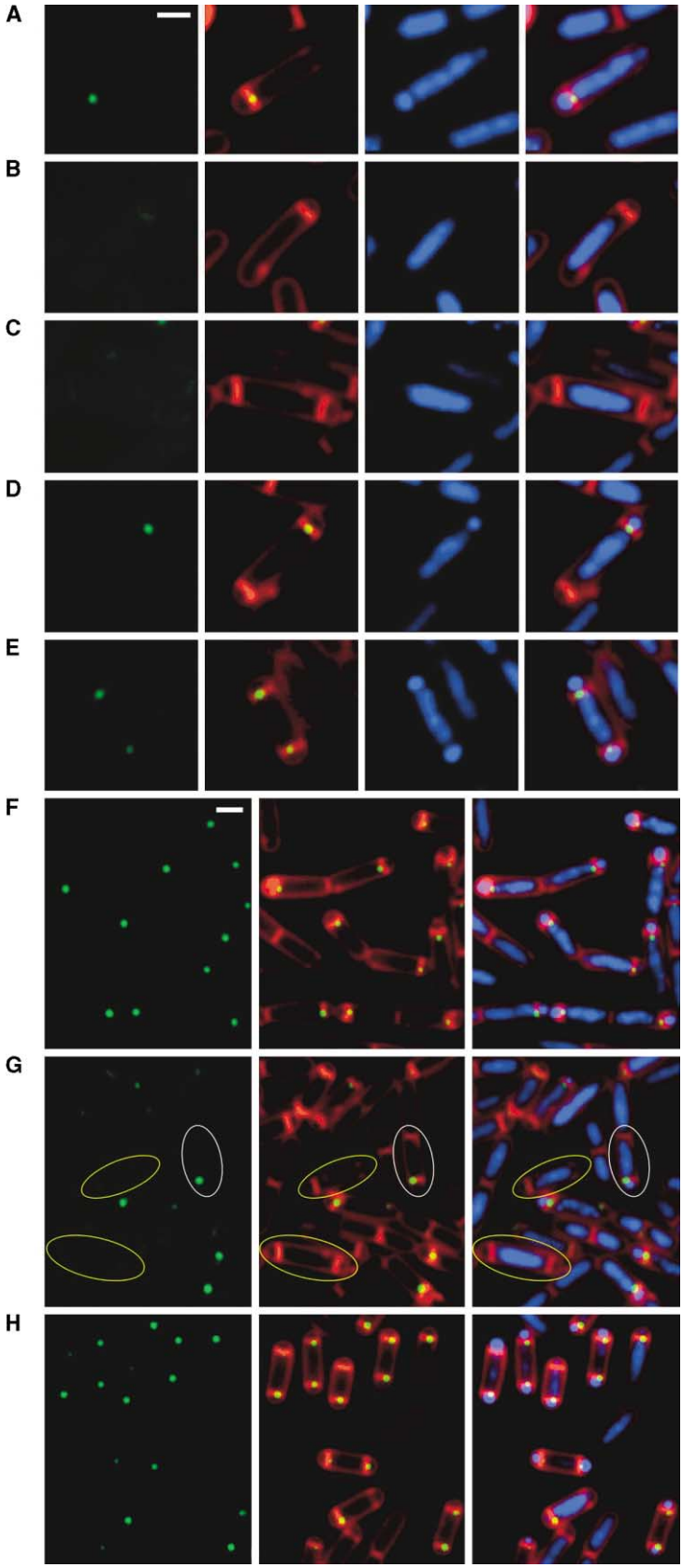

Figure 1. DNA Translocase Localization Depends on DNA Trapping

Sporulating cells of strains producing SpoIIIE-GFP (green) were stained with FM4-64 (red) and with DAPI (blue) and examined by fluorescence microscopy at hour 3 of sporulation (30°C) (fluorescence microscopy was carried out as described previously [22]). Shown are sporangia from: (A and F) a SpoIIIE-GFP-producing, *racA*$^+$ strain (SB234), (B-D and G) a SpoIIIE-GFP-producing, *racA* mutant strain (SB237), and (E and H) a SpoIIIE-GFP-producing *racA*$^+$ strain that contained a mutation (Δ*spoIIAC*) that allowed septa to form at both poles (SB243). (A-E) The left-hand column shows fluorescence from SpoIIIE-GFP, the second column from the left shows an overlay of the SpoIIIE-GFP signal with FM4-64, the third column from the left shows the DNA stained with DAPI, and the right-hand column shows an overlay of fluorescence from SpoIIIE-GFP with that from FM4-64 and DAPI staining. (F-H) The left-hand column shows fluorescence from SpoIIIE-GFP, the middle column shows an overlay of the SpoIIIE-GFP signal with FM4-64, and the right-hand column shows an overlay of fluorescence from SpoIIIE-GFP with that from FM4-64 and DAPI staining. The yellow ovals in (G) identify bipolar sporangia of the *racA* mutant that failed to trap DNA in both forespore compartments and therefore lacked foci from SpoIIIE-GFP. The white oval in (G) identifies a mutant sporangium that trapped DNA in the forespore and, as a result, exhibited a SpoIIIE-GFP focus in the septum. In cases (B, C, and G) in which a fluorescence SpoIIIE-GFP focus was not observed (due to absence of DNA transversing a septum), a faint fluorescence signal from SpoIIIE-GFP that colocalized with the cell membrane was observed. This signal was difficult to capture and cannot be readily seen in the fluorescence images shown here. The scale bars correspond to 1 μm.

Table 1. Frequency of SpoIIIE-GFP Focus Formation

	ΔracA	*ΔspoIIAC*
	2%	87%
	<1%	13%
	36%	<1%
	58%	<1%
others	4%	<1%

SpoIIIE-GFP-producing cells of a *racA* mutant (SB237) and a *spoIIAC* mutant (SB243) were stained with the membrane stain FM4-64 and with DAPI and examined by fluorescence microscopy at hour 3 of sporulation (30°C). (Fluorescence microscopy was carried out as described previously [22]). At least 250 sporangia were scored for each strain. The table lists the percentages of sporangia that exhibited the indicated patterns of SpoIIIE-GFP localization (open circles) observed with respect to chromosome trapping (grey) at both poles, at one pole, or at neither pole of the sporangia.

second chance in DNA trapping, resulting in two types of bipolar sporangia: those that were successful in capturing DNA in the second round of division and those that lack DNA in both polar compartments [6]. The localization of SpoIIIE-GFP was especially revealing in the case of such bipolar mutant sporangia. When one of the two polar compartments contained DNA, a SpoIIIE-GFP focus was present in the polar septum that had trapped a chromosome, but not in the distal septum that had not (Figure 1D, Table 1). When neither polar septum had trapped a chromosome, then neither polar septum exhibited a fluorescent focus (Figures 1C and 1G and Table 1). As a control, we examined SpoIIIE-GFP localization in sporangia that were mutant for the pathway that normally prevents the formation of a second polar septum in wild-type cells (due to a mutation in the sporulation gene *spoIIAC*) [3]. Such a "disporic" mutant forms septa at both poles, and each polar septum traps a chromosome. The results show that a high proportion of both polar septa of such disporic sporangia exhibited a fluorescent focus (Figures 1E and 1H and Table 1). The minor class of disporic sporangia that contained only one focus (13%, Table 1) were ones that had either finished translocating DNA or had not yet started pumping DNA into one of the polar compartments.

Taken together, these results show that SpoIIIE-GFP localization depends on DNA trapping and are consistent with the idea that molecules of the DNA translocase assemble around the trapped chromosome where it crosses the septum. Our findings also suggest that at least some DNA must be trapped in the forespore at the time of polar septation in order to initiate the assembly of the translocase and hence for a chromosome to be subsequently pumped into the forespore. An additional conclusion from this work is that the direction of DNA translocation (into the forespore) is not determined by, or dependent upon, the RacA-mediated attachment of the origin region to the cell pole or by specific DNA sequences located in the region of the chromosome that normally crosses the septum in wild-type sporulating cells. Rather, and in keeping with the proposal of Sharp and Pogliano [11], it is likely that the orientation of SpoIIIE molecules in the septum dictates the direction of DNA movement (see also Chary and Piggot [12] for an alternative point of view on the basis for directionality). As pointed out by Sharp and Pogliano [11], the number of SpoIIIE molecules is likely to be approximately eight times greater in the mother cell than in the forespore compartment (due to the larger size of the former), and this asymmetry in protein distribution might contribute to the polarity of SpoIIIE molecules in the septum (with severalfold more molecules being inserted from the mother cell face of the septum than from the forespore face and thereby determining the direction of translocation). Sharp and Pogliano [13] have suggested that the polar-localized proteins MinC and MinD also contribute to biasing the assembly of the translocation channel to the mother cell face of the septum.

In extension of these ideas, we propose that molecules of SpoIIIE are inserted into and throughout the cytoplasmic membrane of the predivisional sporangium. Next, when the polar septum is formed around the axial filament, SpoIIIE molecules diffuse into the septum where they coalesce around the trapped chromosome, assembling into a channel through which DNA will be translocated (Figure 2). The cytoplasmic domain of a related protein TrwB (involved in DNA conjugation in *E. coli*) has been crystallized and shown to form a hexameric ring [14]. Thus it is possible that the SpoIIIE channel is similarly composed of a single ring or a series of stacked rings (Figure 2). We assume that the bright focus observed with SpoIIIE-GFP contains most of the SpoIIIE molecules in the cell (based on the intensity of the signal and the significant reduction in peripheral membrane signal). Therefore, we favor the model in which the channel is composed of a series of rings rather than a single ring with six molecules. The stacked rings could surround both of the double helices that transverse the septum or, alternatively, SpoIIIE might assemble into two adjacent channels, each separately trapping a single double helix.

As reported previously [11] SpoIIIE-GFP can sometimes be seen as colocalizing with the polar division septum early in sporulation. We have also observed this localization pattern both in wild-type cells and in RacA mutant cells in the presence and the absence of DNA transversing the septum (data not shown). These observations raise the possibility that association with the cytokinetic ring of the division machinery is an intermediate step in the formation of the DNA translocation channel and that the channel is formed by constriction of the cytokinetic ring. Conceivably, the channel is assembled through ring constriction in a manner that does not depend on DNA but is unstable in the absence of DNA (and hence would not have been detected when chromosomal DNA failed to transverse the septum). We note, however, that association with the division machinery is not essential for the proper localization of SpoIIIE-GFP, as cells engineered to produce the SpoIIIE-GFP after polar septation exhibited normal-looking foci of

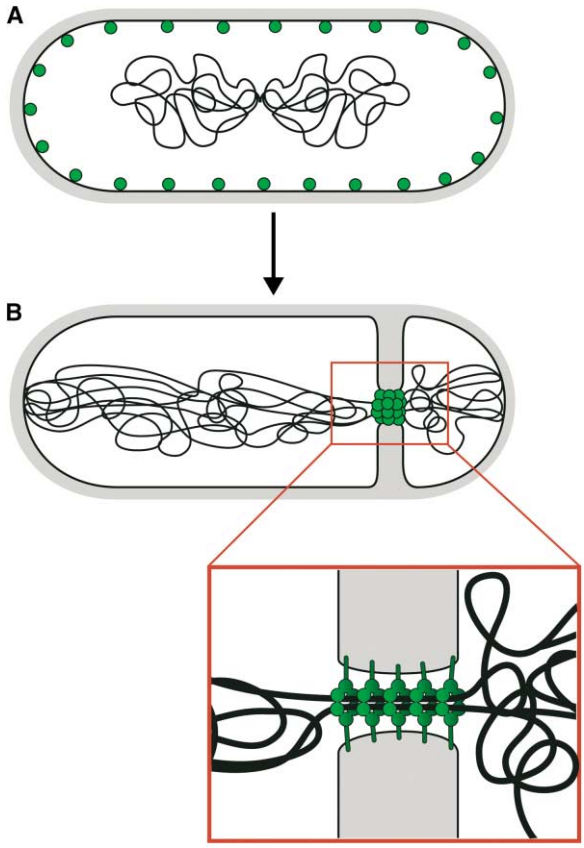

Figure 2. A Model for the Assembly of SpoIIIE DNA Translocation Channel

The cartoons show the proposed stages in assembly of the SpoIIIE DNA translocation channel. (A) shows a predivisional sporangium in which SpoIIIE (green) is inserted throughout the cytoplasmic membrane. (B) shows a postdivisional sporangium with a fully elongated axial filament that reaches the cell poles. The SpoIIIE molecules diffuse through the membrane and coalesce around the trapped chromosome to form the translocation channel. The inset is an enlargement of the channel region in which, as hypothesized, SpoIIIE localizes as a series of staked hexameric rings.

the fusion protein and were able to carry out DNA translocation [11].

The DNA translocase functions conspicuously in sporulation, but SpoIIIE is also present in growing cells when it is required for viability under circumstances in which the coupling of chromosome partitioning and division is perturbed [15–17]. For example, a *spoIIIE* mutation causes a synthetic lethal phenotype when combined with a mutation of the gene for SMC (structural maintenance of the chromosome), which is needed for proper compaction of the chromosome and efficient chromosome segregation [16]. The lack of DNA compaction in an SMC mutant could cause DNA to become aberrantly caught in the division septum, resulting in lethality unless the trapped DNA is removed by the action of the SpoIIIE DNA translocase.

SpoIIIE-like DNA translocases are not restricted to spore-forming bacteria, and most species of bacteria contain at least one member of the SpoIIIE family (*B. subtilis* has at least one additional homolog). For example, *E. coli* contains a homolog of SpoIIIE called FtsK that seems to be involved in linking chromosome segregation to cell division [18]. FtsK is required for proper chromosome segregation and for resolving chromosome dimers resulting from DNA replication [19–21]. Like SpoIIIE, however, FtsK is also a DNA-tracking enzyme [21], and in this capacity the *E. coli* protein is thought to provide a backup mechanism that salvages failures in chromosome portioning in which part of the chromosome is caught in the division septum. Based on our current findings, it is tempting to anticipate that DNA trapping is a general mechanism for the assembly and localization of DNA translocation channels created by FtsK and other SpoIIIE-like proteins.

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References

1. Ryter, A., Schaeffer, P., and Ionesco, H. (1966). Classification cytologique, par leur stade de blocage, des mutants de sporulation de *Bacillus subtilis* Marburg. Ann. Inst. Pasteur (Paris) *110*, 305–315.
2. Webb, C.D., Teleman, A., Gordon, S., Straight, A., Belmont, A., Lin, D.C., Grossman, A.D., Wright, A., and Losick, R. (1997). Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. Cell *88*, 667–674.
3. Piggot, P.J., and Losick, R. (2001). Sporulation genes and intercompartmental regulation. In *Bacillus subtilis* and Its Closest Relatives: From Genes to Cells. A.L. Sonenshein, J.A. Hoch, and R. Losick, eds. (Washington, D.C.: ASM Press), pp. 483–517.
4. Wu, L.J., and Errington, J. (1994). *Bacillus subtilis* spoIIIE protein required for DNA segregation during asymmetric cell division. Science *264*, 572–575.
5. Wu, L.J., and Errington, J. (1997). Septal localization of the SpoIIIE chromosome partitioning protein in *Bacillus subtilis*. EMBO J. *16*, 2161–2169.
6. Ben-Yehuda, S., Rudner, D.Z., and Losick, R. (2003). RacA, a bacterial protein that anchors chromosomes to the cell poles. Science *299*, 532–536.
7. Errington, J., Bath, J., and Wu, L.J. (2001). DNA transport in bacteria. Nat. Rev. Mol. Cell Biol. *2*, 538–545.
8. Donachie, W.D. (2002). FtsK: Maxwell's Demon? Mol. Cell *9*, 206–207.
9. Bath, J., Wu, L.J., Errington, J., and Wang, J.C. (2000). Role of *Bacillus subtilis* SpoIIIE in DNA transport across the mother cell-prespore division septum. Science *290*, 995–997.
10. Sharp, M.D., and Pogliano, K. (1999). An *in vivo* membrane fusion assay implicates SpoIIIE in the final stages of engulfment during *Bacillus subtilis* sporulation. Proc. Natl. Acad. Sci. USA *96*, 14553–14558.
11. Sharp, M.D., and Pogliano, K. (2002). Role of cell-specific SpoIIIE assembly in polarity of DNA transfer. Science *295*, 137–139.
12. Chary, V.K., and Piggot, P.J. (2003). Postdivisional synthesis of the Sporosarcina ureae DNA translocase SpoIIIE either in the mother cell or in the prespore enables Bacillus subtilis to translocate DNA from the mother cell to the prespore. J. Bacteriol. *185*, 879–886.

13. Sharp, M.D., and Pogliano, K. (2002). MinCD-dependent regulation of the polarity of SpoIIIE assembly and DNA transfer. EMBO J. *21*, 6267–6274.
14. Gomis-Ruth, F.X., Moncalian, G., Perez-Luque, R., Gonzalez, A., Cabezon, E., de la Cruz, F., and Coll, M. (2001). The bacterial conjugation protein TrwB resembles ring helicases and F1-ATPase. Nature *409*, 637–641.
15. Sharpe, M.E., and Errington, J. (1995). Postseptational chromosome partitioning in bacteria. Proc. Natl. Acad. Sci. USA *92*, 8630–8634.
16. Britton, R.A., and Grossman, A.D. (1999). Synthetic lethal phenotypes caused by mutations affecting chromosome partitioning in *Bacillus subtilis*. J. Bacteriol. *181*, 5860–5864.
17. Lemon, K.P., Kurtser, I., and Grossman, A.D. (2001). Effects of replication termination mutants on chromosome partitioning in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA *98*, 212–217.
18. Begg, K.J., Dewar, S.J., and Donachie, W.D. (1995). A new *Escherichia coli* cell division gene, *ftsK*. J. Bacteriol. *177*, 6211–6222.
19. Liu, G., Draper, G.C., and Donachie, W.D. (1998). FtsK is a bifunctional protein involved in cell division and chromosome localization in *Escherichia coli*. Mol. Microbiol. *29*, 893–903.
20. Steiner, W., Liu, G., Donachie, W.D., and Kuempel, P. (1999). The cytoplasmic domain of FtsK protein is required for resolution of chromosome dimers. Mol. Microbiol. *31*, 579–583.
21. Aussel, L., Barre, F.X., Aroyo, M., Stasiak, A., Stasiak, A.Z., and Sherratt, D. (2002). FtsK Is a DNA motor protein that activates chromosome dimer resolution by switching the catalytic state of the XerC and XerD recombinases. Cell *108*, 195–205.
22. Ben-Yehuda, S., and Losick, R. (2002). Asymmetric cell division in *B. subtilis* involves a spiral-like intermediate of the cytokinetic protein FtsZ. Cell *109*, 257–266.