Phage ϕ29 protein p1 promotes replication by associating with the FtsZ ring of the divisome in Bacillus subtilis

David Ballesteros-Plaza, Isabel Holguera, Dirk-Jan Scheffers, Margarita Salas, and Daniel Muñoz-Espín

During evolution, viruses have optimized the interaction with host factors to increase the efficiency of fundamental processes such as DNA replication. Bacteriophage ϕ29 protein p1 is a membrane-associated protein that forms large protofilament sheets that resemble eukaryotic tubulin and bacterial filamenting temperature-sensitive mutant Z protein (FtsZ) polymers. In the absence of protein p1, phage ϕ29 DNA replication is impaired. Here we show that a functional fusion of protein p1 to YFP localizes at the medial region of Bacillus subtilis cells independently of other phage-encoded proteins. We also show that ϕ29 protein p1 colocalizes with the B. subtilis cell division protein FtsZ and provide evidence that FtsZ and protein p1 are associated. Importantly, the midcell localization of YFP-p1 was disrupted in a strain that does not express FtsZ, and the fluorescent signal was distributed all over the cell. Depletion of penicillin-binding protein 2B (PBP2B) in B. subtilis cells did not affect the subcellular localization of YFP-p1, indicating that its distribution does not depend on septal wall synthesis. Interestingly, when ϕ29 protein p1 was expressed, B. subtilis cells were about 1.5-fold longer than control cells, and the accumulation of ϕ29 DNA was higher in mutant B. subtilis cells with increased length. We discuss the biological role of p1 and FtsZ in bacterial enlargement.

NA replication of bacterial viruses seems to occur at specific intracellular locations, similar to the replication of DNA from viruses infecting eukaryotic cells. The use of organizing structures of the host constitutes a general viral mechanism to enhance the efficiency of the replication process. Over the last 40 years, different lines of evidence have indicated that the bacterial membrane provides a framework to support replication of distant viral genomes (1–3), thus compartmentalizing this fundamental biological process. In this sense, it has been shown that DNA replication of bacteriophage ϕ29 occurs in association with the Bacillus subtilis membrane (4–6).

B. subtilis phage ϕ29 is one of the best-characterized phages and serves as a model for the study of DNA replication both in vitro and in vivo. The genome of ϕ29 consists of a linear double-stranded DNA with a terminal protein (TP) covalently linked at each 5′ end, and is replicated by a protein-primed mechanism (7, 8) (see Fig. S1 for details). In vitro ϕ29 DNA replication starts with the formation of a heterodimer between the ϕ29 DNA polymerase and a free TP molecule (primer TP) that recognizes the replication origins located at both ends of the viral genome. The DNA polymerase then catalyzes the formation of a covalent linkage between dAMP and the hydroxyl group of serine232 of the primer TP. Replication is coupled to strand displacement, and continuous elongation by the DNA polymerase from both DNA ends gives rise to the formation of replication intermediates that finally converge in the generation of two full-length replicated ϕ29 genomes.

The organization of the phage DNA replication machinery inside the cell has also been studied. Once ϕ29 infection takes place, the N-terminal DNA binding domain of the TP directs the subcellular localization of the phage genome and the TP/DNA polymerase heterodimer at the bacterial nucleoid (9). Later, at middle infection times, the ϕ29 DNA replication machinery is reorganized adopting a peripheral helix-like configuration close to the cell membrane (6, 10).

ϕ29 protein p1 (85 amino acids) is associated with the bacterial membrane and has been proposed to play a role in the organization of ϕ29 DNA replication by providing an anchoring site for the replication machinery (5, 11). Accordingly, in vivo ϕ29 DNA replication was shown to be significantly reduced when nonsuppressor B. subtilis cells were infected with mutant phage sus1(ϕ29), which does not synthesize protein p1 (5). In addition, protein p1 assembles into membrane-associated multimeric structures in vivo (12) and has the capacity to form long protofilaments arranged as bidimensional sheets in vitro (13). Interestingly, these structures resemble protofilament sheets formed by eukaryotic tubulin, the main component of the microtubules, and by filamenting temperature-sensitive mutant Z protein (FtsZ) (14), a prokaryotic homolog of eukaryotic tubulin constituting the most critical component of the bacterial divisome machinery.

During bacterial cell division, FtsZ is the first protein that localizes to the divisome site, and the resulting FtsZ ring (Z ring) functions as a scaffold to recruit other proteins that will produce a new cell wall to separate the future daughter cells (15). FtsZ polymers in a GTP-dependent manner (16), and once the Z ring is assembled, it remains at a midcell position (17). The precise medial localization of the Z ring is strictly regulated spatially and temporally and depends on both the Min system and nucleoid occlusion (18, 19). In B. subtilis, assembly of the divisome occurs by a two-step mechanism (20). There are four early divisome components (FtsA, EzrA, Zapa, and SefP) that interact directly with FtsZ, controlling the site of cell wall synthesis. The later-assembling proteins (FtsL, FtsW, DivB, DivC, and PBP2B) have a single transmembrane span and an extensive extracellular domain, and are involved in septal wall formation.

Here we show that ϕ29 protein p1 localizes at midcell positions in B. subtilis and colocalizes with FtsZ. In fact, protein p1 and FtsZ are associated and, accordingly, the localization of protein p1 is completely disrupted in a strain that does not express FtsZ. By preventing PBP2B synthesis we provide evidence that the recruitment of protein p1 to the divisome machinery is independent on septal wall synthesis and hence, it occurs at early stages of the divisome assembly. Interestingly, expression of protein p1 in B. subtilis cells gives rise to an increased cellular...
length. We find a direct correlation between *B. subtilis* cellular length and the accumulation of ϕ29 DNA and discuss the biological role of protein p1 during the infective cycle.

**Results**

**Phage ϕ29 Protein p1 Colocalizes with the *B. subtilis* Z Ring Independently of Other Phage-Encoded Proteins.** As a first approach to determine the subcellular distribution of ϕ29 protein p1 in vivo, we engineered a *B. subtilis* strain (DBP-001) containing a xylose-inducible fusion of yfp to the phage gene 1 cloned at the chromosomal amyE locus. The fusion protein obtained, YFP-p1, was shown to be functional as revealed by complementation experiments with the ϕ29 mutant phage sus1(629) (Fig. S2). Localization of YFP-p1 was analyzed by fluorescence microscopy in *B. subtilis*-infected cells. Fig. 1A shows that, under a ϕ29 WT infection (i.e., in the presence of phage-encoded proteins), YFP-p1 localized at midcell positions (see arrow and Fig. IE, top part) displaying a conformation reminiscent to that of the divisome machinery. Once a mature divisome is formed, the ingrowth of the cell wall and cytoplasmic membrane produced a complete septum separating the progenitor cell into two daughter cells, where YFP-p1 remained associated (see arrowhead). To assess the distribution pattern of protein p1 at physiological conditions, we infected *B. subtilis* cells with WT phage ϕ29 and analyzed the subcellular localization of the protein by immunofluorescence (IF) techniques (Fig. 1B). In agreement with the results obtained with the YFP fusion, WT protein p1 was observed to localize at the cell center, where the bacterial divisome is presumably organized between the segregating chromosomes stained with DAPI (see arrows). At the time of cytokinesis, protein p1 remained associated with the constriction site and stayed associated with the new cell pole for some time (see arrowheads). We next analyzed the distribution pattern of YFP-p1 in the absence of other phage-encoded proteins (i.e., in noninfected live *B. subtilis* cells). Fig. 1C shows that YFP-p1 was present in a midcell location at divisome (see arrow and Fig. IE, middle part) and constriction sites (see arrowhead), revealing that its distribution is independent of other ϕ29-encoded proteins.

To further show that ϕ29 protein p1 localizes at the bacterial divisome assembling site, we constructed a *B. subtilis* strain (DBP-002) simultaneously expressing functional FtsZ-CFP and YFP-p1 fusions from isopropyl-β-D-thiogalactopyranoside (IPTG)- and xylose-inducible promoters, respectively (*Materials and Methods*). Analysis by Western blotting showed that when the culture was supplemented with IPTG and xylose, bands corresponding to the expected size for FtsZ-CFP and YFP-p1 were detected (Fig. S3). The results shown in Fig. 1D revealed that, in noninfected *B. subtilis* cells, both FtsZ and protein p1 fusions follow a similar path in a midcell location (Fig. IE, bottom part), and superimposition of the CFP and YFP fluorescent signals showed that both proteins colocalize.

Together, the results indicate that ϕ29 protein p1 is localized at the Z ring site independently of the expression of other viral proteins.

**Midcell Localization of YFP-p1 Is Disrupted in the Absence of FtsZ and Does Not Depend on Septal Wall Synthesis.** To determine whether the subcellular localization of ϕ29 protein p1 depends on FtsZ, we used a *B. subtilis* strain with IPTG-conditional synthesis of FtsZ (1801) (21) that was genetically modified to express YFP-p1 in a xylose-dependent manner (DBP-005). As observed in Fig. 2A, in the WT background (i.e., when FtsZ was expressed by adding IPTG), YFP-p1 localized at midcell position and future division sites. In contrast, in the absence of FtsZ, *B. subtilis* cells exhibited a filamentous morphology as a consequence of a failure in septum formation and cell division, and YFP-p1 was distributed throughout the cell, sometimes in combination with weak punctate patterns.

In *B. subtilis*, assembly of the divisome machinery occurs by a two-step mechanism in which the early-assembling proteins are directly recruited to the Z ring by FtsZ and, after a delay, are followed by the concerted and independent association of the later-assembling proteins (20). Among the later-assembling components, PBP2B is involved in septal peptidoglycan synthesis and deletion of PBP2B blocks cell division resulting in bacterial filamentation (22). The above experiments suggested that

---

**Fig. 1.** Subcellular localization of phage ϕ29 protein p1. (A) YFP, membrane staining, and merged images of typical *B. subtilis* cells expressing xylose-induced YFP-p1 fusion protein after ϕ29 infection at a MOI of 5. Cells were analyzed 30 min after xylose and ϕ29 addition. YFP signal is false-colored green. (B) Phage ϕ29-infected *B. subtilis* cells were analyzed by immunofluorescence (IF) using anti-p1 antibodies (green) 20 and 30 min after infection. Bright field, protein p1, DAPI (red colored), and merged images are shown. (C) YFP, membrane staining, and merged images of typical noninfected *B. subtilis* cells expressing xylose-induced YFP-p1 fusion protein. Cells were analyzed 30 min after xylose addition. YFP signal is false-colored green. (D) Bright field, YFP, CFP, and merged images of typical *B. subtilis* cells expressing xylose-induced YFP-p1 and IPTG-induced FtsZ-CFP fusion proteins analyzed 30 min after xylose and IPTG addition. For clarity, YFP and CFP are false-colored green and red, respectively. (Scale bar, 2 µm.) (E) Quantitative analysis of the results. Percentage of cells expressing YFP-p1 with midcell localization in infected and noninfected cells or percentage of cells with YFP-p1/FtsZ-CFP midcell colocalization, when indicated.
recruitment of protein p1 to the divisome site occurs at early assembly stages, as protein p1 was detected in a portion of the cells where the cytoplasmic membrane was not yet invaginated (Figs. 1 and 2A, see arrows). To determine whether septal wall synthesis is required for protein p1 recruitment at midcell locations, we made use of B. subtilis strain 804 (22), in which FtsZ and protein p1 associates with strains DBP-003 (B. subtilis) expressing promoter, B. subtilis to Lys phage ϕB. subtilis

Protein p1 associates with strain (168His) expressing promoter, B. subtilis Subcellular localization of phage B. subtilis ϕ

July 23, 2013

PNAS

−

strain 168

| protein p1 was observed in whole cell lysates from infected cells expressing FtsZ and His-tagged FtsZ (lanes 1 and 3). After chemical cross-linking, protein p1 and p1 oligomers were detected in whole cell lysates of infected cells expressing both FtsZ and His-tagged FtsZ (lanes 7 and 9). Importantly, protein p1 was only detected in the eluted fraction corresponding to the purified His-tagged FtsZ complexes (cf. lanes 4 and 6). This result shows that FtsZ and protein p1 are associated in a complex.

To test whether B. subtilis FtsZ and ϕ29 protein p1 interact directly, we performed in vitro cross-linking assays. Protein p1 has an amphiphilic nature (12) and hence we deleted 11 C-terminal residues spanning Val29 to Lys32 to obtain a soluble variant of the protein (p1ΔC11) (Materials and Methods). After disuccinimidyl suberate (DSS) treatment, products corresponding to the expected size for FtsZ–p1ΔC11 complexes were detected using both anti-FtsZ and anti-p1 antibodies (Fig. 3B, lanes 6 and 12; see arrows). The migrating products were not observed when FtsZ and p1ΔC11 were separately cross-linked (lanes 4, 5, 10, and 11). This result suggests that FtsZ and protein p1 interact directly.

Synthesis of Protein p1 Increases B. subtilis Cellular Length and the Bacterial Enlargement Promotes Viral DNA Accumulation. Although the absence of protein p1 impairs ϕ29 DNA synthesis in B. subtilis (5), little was known about its biological function. To gain insight into the role of protein p1 in live cells, we engineered a B. subtilis strain expressing WT protein p1 under an IPTG-inducible Pspac promoter (DBP-005) and analyzed the bacterial morphology by using the N-(3-trithylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM 4-64) membrane dye. As a control, we used in parallel a strain expressing CFP under the same promoter (strain DM-024). Western blot analyses showed that the amount of protein p1 expressed by the strain DBP-005 was similar to that seen in infected B. subtilis Δspo0A (Fig. S4). In the absence of IPTG, the bacterial cells displayed a normal size with a mean cell length of 3.78 μm (Fig. 4A; Table S1).

Fig. 2. Subcellular localization of phage ϕ29 protein p1 in the absence of the B. subtilis divisome proteins FtsZ or PBPSB. YFP, membrane staining, and merged images of B. subtilis strains DBP-003 (A) and DBP-004 (B) expressing a xylose-induced YFP-p1 fusion protein were acquired 30 min after xylose addition. (A) Effect of the absence of protein FtsZ on the localization of protein p1. (B) Effect of the absence of PBPSB on the localization of protein p1. YFP signal is false-colored green. (Scale bar, 2 μm.) Graphics represent a quantitative analysis of the results.

Fig. 3. Protein p1 associates with B. subtilis FtsZ. (A) Total lysates (Lysate), formaldehyde-treated and reverted eluates (+FA-R Eluate), and formaldehyde-treated and reverted total lysates (+FA-R Lysate) from noninfected (−) or from ϕ29 sus14 (1242) infected (+) cells were analyzed by Western blot using anti-p1 antibodies (Materials and Methods). For simplicity, B. subtilis strain 168His, which expresses WT protein FtsZ, and the B. subtilis strain 168His, which expresses His-tagged FtsZ, were denominated FtsZ and FtsZHis, respectively. (B) The purified protein p1ΔC11 (8.8 kDa) was incubated with B. subtilis protein FtsZ (40 kDa) in the presence of 5 mM MgSO4. Then, GTP was added to a final concentration of 1 mM, and DSS was used as the cross-linking reagent (Materials and Methods). Arrows indicate complexes between FtsZ and p1ΔC11. Numbers indicate the molecular weight in kilodaltons.
viral DNA at the end of the phage infective cycle. As an internal control, we determined that strains DBP-006 and DBP-007 are infected similarly by phage ϕ29 (Fig. S5; Table S3). Fig. 4C and Table S4 show the amount of normalized viral DNA (μg/mL) at 40 and 50 min postinfection, before bacterial lysis. The intracellular accumulated ϕ29 DNA increased ~35% and 31% at 40 and 50 min, respectively, in the ezrA(R510D) mutant compared with the WT strain. This result establishes a direct correlation between cellular length and ϕ29 DNA accumulation.

**Discussion**

During their infective cycle, bacteriophages are proficient in producing high numbers of progeny within short periods of time. To do so, and due to the reduced size of their genomes, they are capable of using some cellular functions and structures of their hosts for their own benefit. Thus, phage DNA replication must be efficiently organized to allow simultaneous amplification of multiple viral genomes in a narrow time window. In the case of bacteriophage ϕ29, the lytic cycle is completed in about 50 min, generating up to 1,000 phage particles for each single *B. subtilis*–infected cell, and it has been demonstrated that ϕ29 takes advantage of some cellular components during viral DNA replication, such as the bacterial cytoskeleton, termed the MreB cytoplasmic membrane (4–6, 10). In agreement with the production of such elevated amounts of phage progeny, the number of ϕ29 DNA replication proteins synthesized in the infected cell is very high. For example, the amount of ϕ29 DNA polymerase increases up to ~1,000 molecules per cell during the infection cycle and the TP up to ~10,000 molecules per cell (5). The intracellular concentration of protein p1 has been determined to increase along the infection cycle, reaching levels about 60-fold higher than those of the phage DNA polymerase (5).

The results of this work, together with those obtained previously, lead us to propose a comprehensive model about the biological role of protein p1 during phage ϕ29 DNA replication in *B. subtilis*–infected cells (Fig. 5). After TP-DNA injection takes place, the viral genome is recruited to the bacterial nucleoid (Fig. 5A, ii) by means of the DNA binding domain of the parental TP. The bacterial nucleoid provides an appropriate environment for both ϕ29 DNA replication and transcription, as it contains high concentrations of deoxy- and ribonucleotides and because the phage needs to make use of the *B. subtilis* RNA polymerase, which has been shown to be located at the bacterial nucleoid (24). The early operon located at the left side of the ϕ29 genome encodes protein p1 and essential components of the ϕ29 DNA replication machinery (7). Once the latter is located at the bacterial nucleoid, the viral genome begins to be replicated and, at the time that cell division is initiated, the assembly of the Z ring (Fig. 5A, iii and B, i) serves as a scaffold for the recruitment of protein p1 (Fig. 5A, iv and v). During recruitment of other early-assembling division proteins by FtsZ, protein p1 multimerizes forming bidimensional protofilaments and is organized as a midcell structure (Fig. 5B, ii). Our results suggest an early association of protein p1 along the Z ring, which would be followed by the recruitment of the later-assembling proteins involved in septum formation: FtsL, FtsW, DivIB, DivIC, and PBP2B (Fig. 5B, iii). This early association is based on the fact that protein p1 localization is not altered in cells lacking PBP2B and thus is independent on septal wall synthesis. Moreover, protein p1 is synthesized from the beginning of the ϕ29 infection cycle (5) and associates with FtsZ. At middle infection stages, the phage ϕ29 DNA replication machinery is redistributed in a helix-like configuration along the cell membrane (Fig. 5A, vs). Although protein p1 was initially proposed to play a role in the organization of ϕ29 DNA replication at the bacterial membrane (25), this possibility seems now unlikely due to the fact that it is located to the divisome site during the infection cycle. In fact, it has been shown that in the last stages of viral DNA replication, ϕ29 membrane–protein p16.7 is responsible for attaching phage DNA to peripheral sites by interacting with the bacterial MreB cytoskeleton, which also associates with the cell membrane spanning the entire bacterial length (6).
Growth and division of rod-shaped bacteria such as *B. subtilis* requires a coordinated and sequential switching between two modes of growth that probably compete with each other (26). In growing cells, peptidoglycan is synthesized along the sidewall resulting in bacterial elongation (Fig. 5A, vii). At the time of cell division, the synthesis apparatus switches from sideway peptidoglycan synthesis to division septum synthesis that is followed by cell constriction and disassembly of the divisome components (Fig. 5A, vii). We found increased cellular length in *B. subtilis* cells expressing protein p1. It is tempting to speculate that protein p1 might delay septum formation and/or disassembly of the divisome into its elementary parts, and hence the sidewall precursors would insert peptidoglycan molecules for a longer period before cell division, resulting in daughter cells with an increased length (Fig. 5A, vii–ix). Because the bacterial membrane constitutes the site where the ϕ29 DNA replication machinery is finally redistributed, its expansion by a delayed cell division caused by the association of protein p1 to the bacterial divisome would provide a viral strategy to optimize the production of higher amounts of viral DNA. In agreement with this view, the intracellular accumulation of ϕ29 DNA was significantly affected when *B. subtilis* cells were infected with a mutant phage for gene 1 (5), and the production of viral DNA increased in a strain longer than WT cells (Fig. 4C).

There is compelling evidence that eukaryotic viruses have developed the capacity to take advantage of both actin and tubulin cytoskeleton components. Paradoxically, whereas bacterial cytokinesis is organized by the tubulin homolog FtsZ, the eukaryotic contractile ring is formed by actin. Inversely, whereas the bacterial cytokinetic machine is organized by the actin homolog MrE, the eukaryotic framework depends on tubulin microtubules. It is known that some eukaryotic viruses have learned to impair cytokinesis progression. For instance, the nucleocapsid (N) protein of the severe acute respiratory syndrome coronavirus inhibits cell cytokinesis and proliferation by interacting with human translation elongation factor 1α (EF1α) (27), and the *Rubella* virus replicase protein P90, which interacts with the cytokinesis regulatory protein Citron-K kinase, causes cell cycle arrest and chromosomal aberrations (28). After infecting eukaryotic cells, cytoskeleton-based transport provides to numerous viruses the means to reach their site of replication (normally the nucleus) and generates the route for the newly assembled progeny to leave the infected cell. In addition to actin, many viruses are able to use the microtubules network as tracks to move their components throughout the dense cytoplasm (29). It is worth mentioning that adenoviruses, which similarly to phage ϕ29 replicate their genomes by a protein-primed mechanism, move their capsids in a microtubule-dependent fashion toward and away from the microtubule-organizing center to target the cell nucleus and hence are able to exploit eukaryotic homologs of bacterial FtsZ (30). In a prokaryotic parallelism, it has been recently identified that bacteriophage 201q-2-1 synthesizes a tubulin-like protein, PhuZ, which forms dynamic filaments in vivo that are required for positioning phage DNA within the bacterial cell (31).

Preceding studies have demonstrated the importance of the tubulin homolog FtsZ to orchestrate the assembly of the bacterial cytokinetic machinery and hence to coordinate the bacterial septum formation before cell division. Using *B. subtilis* phage ϕ29 as a model system, we report that the Z ring is hijacked early by the ϕ29 protein p1. Moreover, we provide evidence that protein p1 expression gives rise to an increase in cellular length and that the accumulation of phage DNA is higher in cells with increased size. Finally, we propose a mechanism to optimize the production of large amounts of viral DNA by expanding the surface of the ultimate site of phage ϕ29 DNA replication: the bacterial membrane. These results emphasize that the well-known ability of eukaryotic viruses to use tubulin-like components of their hosts has a precedent in bacterial viruses.

**Materials and Methods**

**Bacterial Strains, Phages, and Growth Conditions.** *Escherichia coli* DH5α and XL1-Blue strains were used for cloning, and *E. coli* strain BL21(DE3) was used for protein expression. To select the cloned plasmids, transformed *E. coli* cells were grown in Luria-Bertani (LB) medium containing 100 μg/mL ampicillin and 1.5% (wt/vol) agar. *B. subtilis* strain 168 was considered the WT strain. Because phage ϕ29 DNA replication is inhibited by SpOA (32), spoOA deletion strains were used when the experiments required phage infection. Bacterial strains used and their relevant characteristics are shown in *Table S5*. WT phage ϕ29,
the gene 1 mutant phage 429 sus1629 (33), or the delayed-lysogeny mutant phage 429 sus141242 (34) (Table S5) was used to infect B. subtilis cells where indicated. The mutation in gene 14 has no effect on phage DNA replication or phage morphogenesis but allows examination of phage protein and DNA localization at late infection times because it has a delayed lysis phenotype.

B. subtilis cells were grown overnight in LB medium supplemented with the required antibiotics: chloramphenicol, erythromycin, kanamycin, neomycin, phleomycin, and spectinomycin (final concentration of 5, 1, 5, 5, 1, and 100 μg/ml, respectively). Also, when required for growth (B. subtilis strains 1801, 804, and variants), 0.5 mM IPTG or 0.5% (wt/vol) xylose was added. Overnight cultures were diluted 1:100 in the same fresh medium and cultured at 37 °C to reestablish exponential growth. When B. subtilis cells were infected with WT 29, 29 sus1629, or 29 sus141242, LB medium was supplemented with 5 mM MgSO4. Expression of YFP-p1 was induced by the addition of xylose to a final concentration of 0.5% (wt/vol). To express FtsZ-CFP, 1 mM of IPTG was used.

B. subtilis cells were transformed by standard procedures, as described previously (35).

Fluorescence Microscopy Experiments. All fluorescent microscopy images were acquired with a laser scanning microscope (Zeiss LSM710 coupled to an inverted AxioObserver) except for colocalization and immunofluorescence photographs, which were taken with a Zeiss Inverted Axiovert200 microscope coupled to a monochrome ccd camera (Hamamatsu C9100-02).

See SI Materials and Methods for details.

Pull-Down Assay. For details on the pull-down assay, see SI Materials and Methods.

In Vitro Cross-Linking. For details on in vitro cross-linking, see si Materials and Methods.


B. subtilis Cellular Length Measurements. Cells were stained with FM 4-64 and photographed with a laser scanning multiphoton microscope (Zeiss LSM710 coupled to an inverted AxioObserver and Zeiss LSM510 coupled to a vertical Axiolimager M1). Cellular length was measured with the FIJI software (ImageJ) and was considered to be the distance from the orthogonal half of one septum up to the orthogonal half of the next adjacent septum. Data were analyzed with GraphPad Prism. See SI Materials and Methods for details.

Quantification of Viral DNA Synthesis. B. subtilis strains DBP-006 and DBP-007 were grown overnight at 37 °C in LB medium supplemented with 5 mM MgSO4 and the required antibiotics (5 μg/ml kanamycin for DBP-006 and 100 μg/ml spectinomycin and 5 μg/ml kanamycin for DBP-007). To reestablish exponential growth, overnight cultures were diluted 1:200 in the same fresh medium and grown at 37 °C. At an OD600 of 0.2, each culture was infected with WT phage 29 at a multiplicity of infection (MOI) of 0.5 and grown at 37 °C. One milliliter of culture was harvested at the indicated times after infection, processed, and analyzed by real-time PCR (SI Materials and Methods).

ACKNOWLEDGMENTS. We thank Dr. Peter Lewis for providing the B. subtilis strain 1057, Dr. Richard Daniel for B. subtilis strains 1801 and 804, Dr. Yoshikazu Kawai for B. subtilis strain 168fts2Hs, and Dr. Petra Levin for B. subtilis mutant strain 141242, and its isogenic WT strain 149(7). This investigation was supported by Grants BFU2011-23645 and Consolider-Ingenio CSD2007-00015 from the Spanish Ministries of Economy and Competitiveness and Science and Innovation, respectively; Nuevos materiales y dispositivos biolucinos cofunded to a Nanociencia Comunidad de Madrid (NOBIMAT-CM) S2909/MA/1507 from the Comunidad Autónoma de Madrid (to M.S.); The Netherlands Organization for Scientific Research (to D.-J.S.); and an institutional grant from Fundación Ramón Areces to the Centro de Biología Molecular “Severo Ochoa.” D.B.-P. was the holder of a Formación de Personal Investigador (FPI) fellowship from the Spanish Ministry of Science and Innovation and of a contract from the Comunidad Autónoma de Madrid. I.H. is the holder of a Formación de Profesorado Universitario (FPU) fellowship from the Spanish Ministry of Education. D.M.-E. was the holder of a Consolider-Ingenio contract.