Molecular Cell, Volume *57*

Supplemental Information

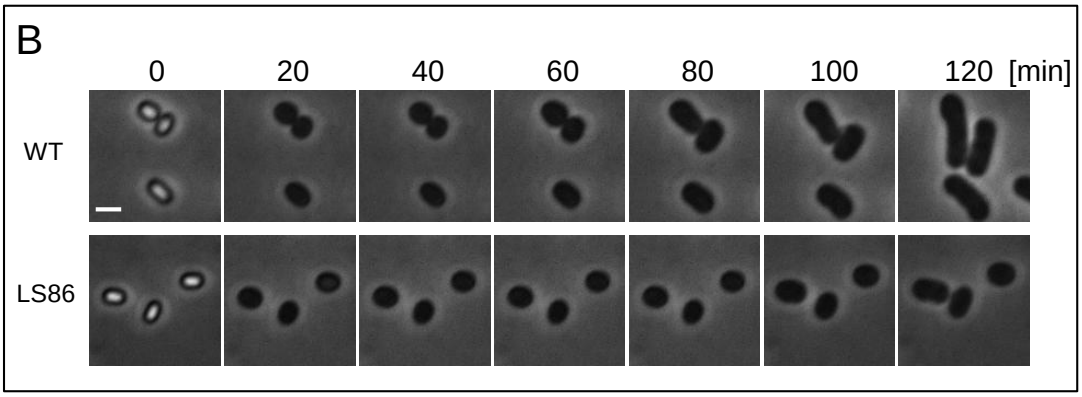
The Molecular Timeline of a Reviving Bacterial Spore

Lior Sinai, Alex Rosenberg, Yoav Smith, Einat Segev, and Sigal Ben-Yehuda

Figure S1

A

Strain	µmoles of L-malate per g of cells/spores
PY79 vegetative cells	Below calibration curve values
PY79 spores	30.5
LS86	30.7



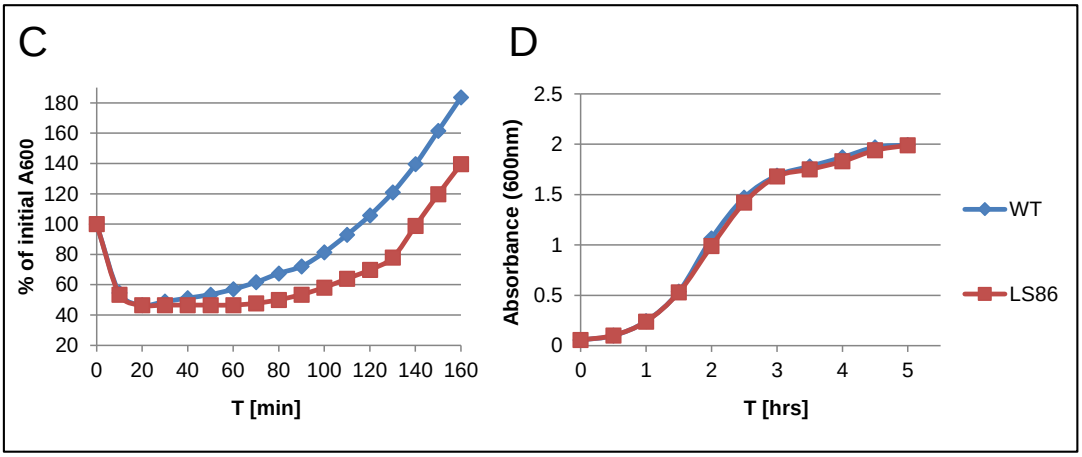
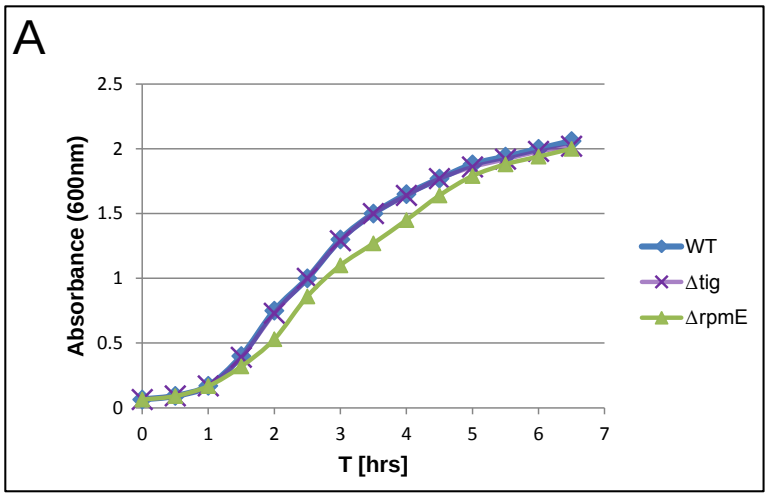
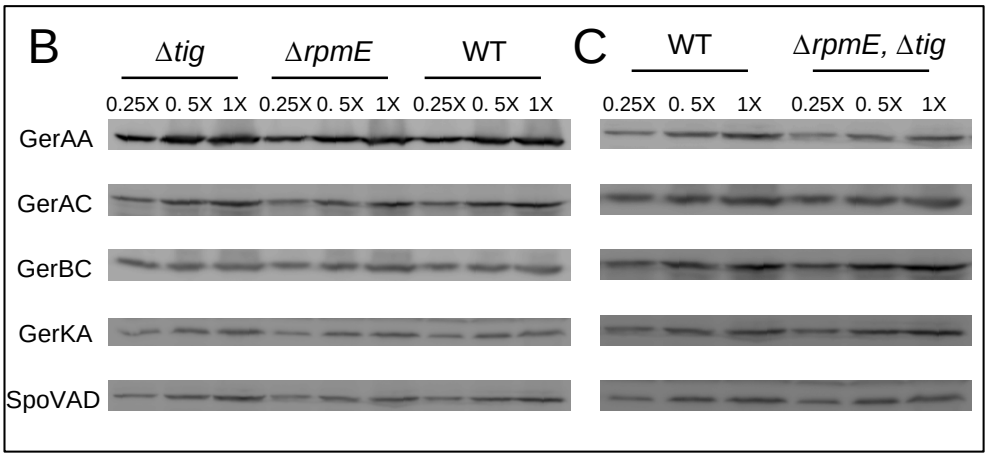


Figure S2





D

Strain	Genotype	Spores/ml
PY79	Wild type	2.71 X 10^8
LS38	*Δtig*	2.59 X 10^8
LS26	*ΔrpmE*	2.11 X 10^8
LS78	*Δtig*, *ΔrpmE*	2.01 X 10^8

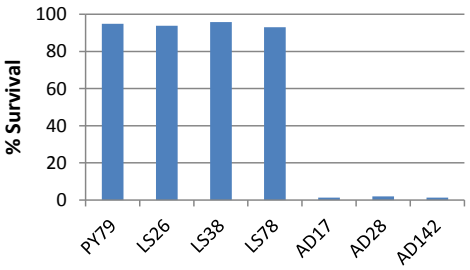


Figure S3

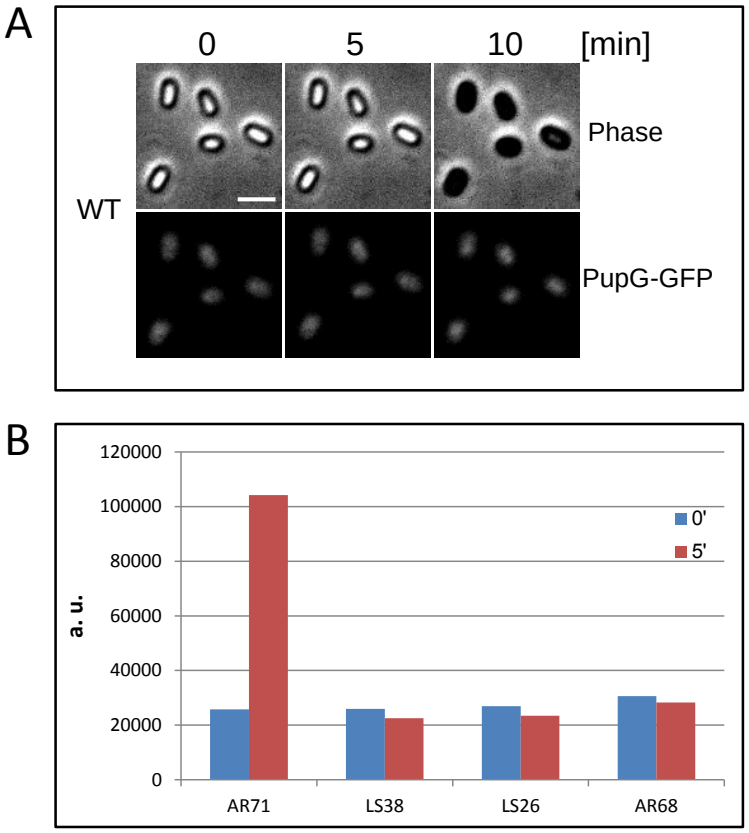

A
0
5
10
[min]
Phase
WT
PupG-GFP
B
120000
100000
80000
60000
40000
20000
0
a. u.
0'
5'
AR71
LS38
LS26
AR68

Figure S1. Spores store malate as a carbon source to energize revival

Related to Figure 2

(**A**) Equal dry weight (4 mg) of PY79 (wild type, WT) spores, LS86 (Δ*maeA,* Δ*malS,* Δ*mleA,* Δ*ytsJ*) spores and WT vegetative cells were lysed, and extracts were subjected to malate dehydrogenase assay (see Supplemental Experimental Procedures). Malate concentration was determined by comparing OD_{340} values to a standard curve of known increasing malate concentrations.

(**B**) Spores of PY79 (wild type, WT) and LS86 (ΔMaeA, ΔMalS, ΔMleA, ΔYtsJ) strains were incubated in revival medium and monitored by time lapse microscopy. Shown are phase contrast images acquired at 20 min intervals. Scale bar represents 1 μm.

(**C**) Spores of PY79 (wild type, WT) and LS86 (ΔMaeA, ΔMalS, ΔMleA, ΔYtsJ) strains were incubated in revival medium and optical density (OD_{600}) was measured at the indicated time points. Data are presented as a fraction of the initial OD_{600} of the phase-bright spores. Decreasing OD_{600} signifies spore germination, and increasing OD_{600} indicates spore outgrowth (Moir and Smith, 1990).

(**D**) PY79 (wild type, WT) and LS86 (ΔMaeA, ΔMalS, ΔMleA, ΔYtsJ) strains were diluted to OD_{600}=0.05 in S7 minimal medium supplemented with amino acids, incubated at 37°C and optical density (OD_{600}) was measured during growth at the indicated time points**.**

Figure S2. Characterization of Δ*tig* and Δ*rpmE* mutant strains

Related to Figure 5

(**A**) PY79 (wild type, WT), LS38 (Δ*tig),* and LS26 (Δ*rpmE*) strains were diluted to OD_{600}=0.05 in LB medium, incubated at 37°C and optical density (OD_{600}) was measured during growth at the indicated time points.

(**B-C**) Equal amounts of protein extracts prepared from spores of PY79 (wild type, WT), LS38 (Δ*tig),* LS26 (Δ*rpmE*) and LS78 (Δ*tig,* Δ*rpmE*) strains were subjected to SDS-PAGE followed by Western blot analysis. Membranes were probed with antibodies against GerAA, GerAC, GerBC, GerKA, and SpoVAD (see Supplemental Experimental Procedures). Dilutions of the different samples were loaded on the same gel for comparison.

(**D**) The indicated strains were induced to sporulate in DSM for 48 hours. Next, cultures were subjected to heat kill treatment (80°C, 30 minutes), serial decimal dilutions were plated on LB agar and colonies were counted after 24 hours. Presented are the averaged numbers of spores / ml from 3 independent biological repeats.
(E) Spores of PY79 (WT), LS26 (Δ*rpmE*), LS38 (Δ*tig),* LS78 (Δ*tig*, Δ*rpmE*), AD17 (*gerE36*), AD28 (Δ*cotE)* and AD142 (*gerE36,* Δ*cotE*) strains were incubated with lysozyme (50 μg/ml) and plated on LB. Percentage of survival was calculated as number of colonies after treatment / number of colonies before treatment.

Figure S3. Analysis of protein synthesis during spore germination
Related to Figure 6

(**A**) Spores of AR68 (*pupG-gfp*) were incubated with L-alanine and followed by time lapse microscopy. Shown are phase contrast (upper panels) and fluorescence (lower panels) images taken at the indicated time points. Images were scaled to the same intensity range. The intensity of a wild-type (PY79) strain, lacking the *gfp* gene, was subtracted from the net average fluorescence intensity. A representative experiment out of 3 independent biological repeats is shown. Scale bars represent 1 μm.
(**B**) Signal from GFP fusion proteins (Figure 6C) was quantified by MetaMorph software (version 7.7, Molecular Devices). Bands were marked and their integrated intensity was determined. For each band, the integrated intensity of a same sized background region was subtracted.

Table S1. The proteomic timeline of spore revival

Related to Figure 2

The proteomic timeline of reviving LS5 (Δ*metE*) spores as determined by BONCAT is provided as a separate Excel file. The analysis is based on 3 independent biological repeats. Only proteins detected in all repeats are displayed.

Table S2. Proteins synthesized during spore revival in the present of transcription inhibitors

Related to Figure 2

Protein	**Function**
PdhA	Pyruvate dehydrogenase (E1 alpha subunit)
PdhB	Pyruvate dehydrogenase (E2 beta subunit)
PdhD	Dihydrolipoamide dehydrogenase
RplU	50S ribosomal protein L21
RplP	50S ribosomal protein L16
RplT	50S ribosomal protein L20
YaaH	Spore peptidoglycan hydrolase
GlnA	Glutamine synthetase
TpiA	Triose phosphate isomerase
YqjE	Putative deacylase
YqhR	Putative integral inner membrane protein
YfnH	Sugar-phosphate cytidylyltransferase

Spores of LS5 (Δ*metE*) strain were incubated in reviving medium, in which methionine was replaced by AHA and supplemented with rifampicin (50 μg/ml) and actinomycin D (50 μg/ml). Samples were collected after 30 minutes, and processed as described in Figure 1A.

Table S3. Lincomycin treatment inhibits spore germination

Related to Figure 4

Treatment	**% of non germinated spores**
Ethanol	0.08% ± 0.03%
Lincomycin in Ethanol	84.5% ± 2.1%
Tetracycline in Ethanol	91.3% ± 2.4%
Lincomycin and Tetracycline in Ethanol	99.4% ± 0.4%
Lincomycin in DDW	0.14% ± 0.05%
Tetracycline in DDW	0.11% ± 0.04%

Spores of PY79 (wild type) were incubated at 37°C for 2 hrs in ethanol with or without lincomycin and/or tetracycline (250 µg/ml). Next, spores were washed with phosphate buffer and incubated with L-alanine for 30 minutes to trigger germination. Samples were analyzed by phase contrast microscopy to determine germination efficiency. Summarized in this table are the averaged percentages of non germinated spores from 4 independent experiments. For each experiment, three phase contrast fields were analyzed and the number of phase-bright and phase-dark spores was determined using manual counting procedure in MetaMorph 7.7 software.

Table S4. Heat resistance of germinating spores

Related to Figure 4 and Figure 5

Strain	Genotype	Treatment prior to germination	% of heat resistant spores
PY79	Wild type	-	3.45% ± 1.1%
LS26	Δ*rpmE*	-	4.7% ± 1.5%
LS38	Δ*tig*	-	5.1% ± 1.8%
LS78	Δ*rpmE*, Δ*tig*	-	5.3% ± 2.0%
PY79	Wild type	Ethanol	3.1% ± 1.2%
PY79	Wild type	Lincomycin-ethanol	4.9% ± 2.1%
PY79	Wild type	Tetracycline-ethanol	3.9% ± 1.7%

Spores of LS26 (Δ*rpmE*), LS38 (Δ*tig),* LS78 (Δ*tig*, Δ*rpmE*) and PY79 (WT) not treated or treated with ethanol with or without lincomycin (250 µg/ml) or tetracycline (250 µg/ml) were incubated with L-alanine for 10 minutes and the number of viable cells was determined. The spores were then incubated at 80°C for 30 minutes and plated on LB. Percentage of heat resistant spores was calculated as number of colonies after heat treatment / number of colonies before heat treatment.

Table S5. The spore germination proteome

Related to Figure 4

The proteome of germinating LS5 (Δ*metE*) spores incubated with L-alanine, AGFK or Ca-DPA, as determined by BONCAT, is provided as a separate Excel file. The analysis is based on 3 independent biological repeats. Only proteins detected in all repeats are displayed.

Table S6. Processes awakening during germination

Related to Figure 4

Process	Ca-DPA	L-ala	AGFK	30 min
Glycolysis	9	9	9	13
Pyruvate dehydrogenase complex	4	4	4	4
Translation elongation factors	3	3	3	3
Utilization of malate	1	2	4	4
Chaperones	4	5	6	8
Ribosomal subunits	3	3	19	47
Electron transport	0	1	6	6
Oxidative and electrophile stress response	1	2	3	3
Transition state regulators	0	1	2	2
RNases	1	1	5	5
Ribosome assembly	0	0	2	2
Purine biosynthesis	0	0	7	15
Transcription machinery	0	0	4	4
Pentose phosphate pathway	1	0	3	4
TCA cycle	0	0	2	4
tRNA Synthetases	0	0	14	17
ATP synthase	0	0	2	9
Proteolysis	0	0	6	10
Overflow metabolism	0	0	2	3
Diglucosyl-diacylglycerol biosynthesis	0	0	2	2
Utilization of peptides	0	0	3	5
Purine salvage and interconversion	0	0	4	4
Nucleotide metabolism	0	0	1	1
Unknown	0	1	2	5
Phosphate metabolism	0	0	1	1
Cofactor biosynthesis	0	0	0	12
Utilization of amino acids	0	0	0	6
Utilization of ribose	0	0	0	3

Biosynthesis of amino acids	0	0	0	5
General stress proteins	0	0	0	3
Acquisition of iron	0	0	0	2
rRNA and tRNA modification and maturation	0	0	0	3
Pyrimidine biosynthesis	0	0	0	2
Total	**27**	**32**	**116**	**217**

A table comparing the number of newly synthesized proteins for each indicated cellular process, as identified by mass spectrometry (data extracted from Table S1 and Table S5), when LS5 (Δ*metE*) spores were germinated with Ca-DPA, L-alanine or AGFK, or incubated in revival medium (30 min).

Table S7. *B. subtilis* strains used in this study

Strain	Genotype	Comments
PY79	Wild type	(Youngman et al., 1984)
LS5	*metE::mls*	The ORF of *metE* was replaced by *mls* gene using a long-flanking-homology PCR with primers 881-884
LS26	*rpmE::kan*	The ORF of *rpmE* was replaced by *kan* gene using a long-flanking-homology PCR with primers 2526-2529
LS38	*tig::kan*	The ORF of *tig* was replaced by *kan* gene using a long-flanking-homology PCR with primers 2530-2533
LS47	*atpA-gfp-cat*	PY79 was transformed with genomic DNA of strain BS23 (Johnson et al., 2004)
LS50	*tig-gfp-spc*	PY79 was transformed with pLS50 (*tig-gfp-spc*)
LS78	*tig::kan, rpmE::spc*	The ORF of *rpmE* in LS38 was replaced by *spc* gene using a long-flanking-homology PCR with primers 2526, 2529, 2536 and 2537
AR68	*pupG-gfp-spc*	PY79 was transformed with pAR68 (*pupG-gfp-spc*)
AR71	*malS-gfp-spc*	PY79 was transformed with pAR71 (*malS-gfp-spc*)
LS80	*tig::kan, malS-gfp-spc*	LS38 was transformed with genomic DNA of strain AR71
LS81	*rpmE::kan, malS-gfp-spc*	LS26 was transformed with genomic DNA of strain AR71
LS82	*tig::kan, metE::mls*	LS38 was transformed with genomic DNA of strain LS5
LS83	*rpmE::kan, metE::mls*	LS26 was transformed with genomic DNA of strain LS5
LS86	*maeA::spc, malS::kan, mleA::tet, ytsJ::mls*	Sequentially, the ORF of *maeA* was replaced by *spc* gene, the ORF of *malS* was replaced by *kan* gene, the ORF of *mleA* was replaced by *tet* gene, and finally the ORF of *ytsJ* was replaced by *mls* gene. All by using a long-flanking-homology PCR using primers, 2538-2541, 2542-2545, 2546-2549 and 2550-2553, respectively
LS100	*rpmE::kan, amyE:: rpmE-cat*	LS26 was transformed with pLS101 (*amyE:: rpmE-spc*)
LS101	*tig::kan, amyE:: tig-cat*	LS38 was transformed with pLS100 (*amyE::tig-spc*)
IB66	*dnaX-gfp-spc*	(Lemon and Grossman, 1998)

SB127	*ezrA-gfp-spc*	(Ben-Yehuda and Losick, 2002)
SB170	*ftsZ-gfp-kan, amyE::ftsAZ*	Laboratory stock
ME141	*tagO-gfp-spc*	(Elbaz and Ben-Yehuda, 2010)
RU97	*gerAA::spc*	The ORF of *gerAA* was replaced by *spc* gene using a long-flanking-homology PCR with primers 2656-2659
AD17	*gerE36*	A gift from Adam Driks (Loyola University Chicago)
AD28	*cotE::cat*	(Driks et al., 1994)
AD142	*gerE36, cotE::cat*	A gift from Adam Driks (Loyola University Chicago)

Long-flanking-homology PCR replacement strategy was based on (Guerout-Fleury et al., 1995) and the resultant PCR product was used to transform PY79. For some of the constructs, Gibson Assembly kit (New England Biolabs) was utilized to assemble the PCR products.

Supplemental Experimental Procedures

Light microscopy

Light microscopy was carried out as described previously (Segev et al., 2012). Briefly, bacterial cells (0.2 ml) were centrifuged and resuspended in 50µl of PBS x 1. Specimens were placed on 1% agarose pads, and visualized using an Axioplan 2 microscope (Zeiss) equipped with a CoolSnap HQ camera (Photometrics, Roper Scientific). For time-lapse revival experiments, spores were placed on 1% agarose pads made of the indicated medium, and incubated in a temperature controlled chamber (Pecon-Zeiss) at 37ºC. For GFP measurements, the intensity of a wild-type (PY79) strain, lacking the *gfp* gene, was subtracted from the net average fluorescence intensity. Samples were photographed using Axio Observer Z1 (Zeiss), equipped with CoolSnap HQII camera (Photometrics, Roper Scientific). System control and image processing were performed using MetaMorph 7.7 software (Molecular Devices).

Spores purification

For BONCAT experiments, spores were purified by water washing as described by Nicholson and Setlow (Harwood and Cutting, 1990). In brief, 400 ml of 48 hrs DSM culture was centrifuged and washed 3 times in 100 ml of DDW. The pellet was resuspended in 80 ml of DDW and kept in 4°C with constant agitation. On subsequent days the suspension was centrifuged once and resuspended in DDW (3 washes a day). Eventually, after 7 days, the pellet contained spores surrounded by a brown layer of cell debris. This layer became very viscous and tight when swirled and therefore easy to remove. The remaining pellet included almost exclusively free spores, as evaluated by phase contrast microscopy.

For microscopy and absorbance read experiments, spores were purified using 3 steps Histodenz gradient. Briefly, a 10 ml 24 hrs DSM culture was washed in DDW and resuspended in 1 ml of 20% Histodenz solution for 30 minutes on ice. Spores were then placed on top of a two step gradient made up from 2 ml 40% histodenz on top of 6 ml 50% histodenz. After centrifugation (90 min, 10,000 RPM, 23°C), a pellet was detected at the bottom of the tube. The pellet contains >99 % pure spore population, as evaluated by phase contrast microscopy.

L-malate determination

L-malate was determined by using an enzymatic method with L-malate dehydrohenase (MDH) as described before (Peleg et al., 1990). Briefly, lyophilized spores and vegetative cells in an equal dry weight (4 mg) were lysed using Fastprep (MP) (6.5, 60 seconds, x3), and extracts were subjected to analysis in reaction mixture containing: hydrazine-glycine buffer (hydrazine, 0.4 M glycine, 0.5 M, pH 9), 2.55 mM NAD+, 10 U of L-malate dehydrogenase (cytoplasmic enzyme from porcine heart; Sigma). The reaction mixtures were incubated for 30 min at 37°C, and the reaction was stopped by boiling samples for 2 min. L-Malic acid was determined by following the formation of NADH at 340 nm. Reactions containing L-malic acid varying in concentration from 24 to 189 μM were use to generate standard curve.

DPA measurements

DPA was assayed by the colorimetric method of (Janssen et al., 1958). Briefly, samples were taken during spore germination, centrifuged, and 4 ml of supernatant fluid was added to 1 ml freshly prepared solution [1% Fe $(NH_4)_2$ $(SO_4)_2$ x 6H20, 1% ascorbic acid in 0.5 M acetate buffer, pH 5.5]. The color developed immediately, and OD_{440} was measured.

Determination of the levels of spore germination proteins

Levels of germinant receptor subunits (GerAA, GerAC, GerBC and GerKA) and SpoVAD were determined based on (Ramirez-Peralta et al., 2012) by Western blot analyses using rabbit antibodies against these proteins and a secondary antibody. Briefly, 125 ODs of spores were incubated at 70°C for 2 hrs in decoating buffer (0.1 M DTT, 0.1 M NaCl, 0.1 M NaOH, 1% SDS) followed by extensive water washes. Samples were treated with 1 mg lysozyme in 0.5 ml TEP buffer (50mM Tris-HCl pH 7.4, 5 mM EDTA) containing 1 mM PMSF, 1μg RNase, 1 μg Dnase I, and 20 μg of $MgCl_2$ at 37°C for 5 min, and then incubated on ice for 20 min. Spores were disrupted using Fastprep (MP) (6.5, 60 seconds, x3), and 100 μL of the lysate was added to 100 μl Laemmli sample buffer containing 55 mM DTT (425 μL BioRad 161-0737 plus 25 μL 1 M DTT) and incubated at 23°C for one hour. Western blot analysis was carried out as described by Ramirez-Peralta et al. (2012).

Determination of the levels of spore germination proteins

Levels of germinant receptor subunits (GerAA, GerAC, GerBC and GerKA) and SpoVAD in the inner membrane fraction of spores were determined by Western blot analyses using rabbit antibodies against these proteins and a secondary antibody as described previously (Ramirez-Peralta et al., 2012).

Western blot analysis of GFP fusion proteins

Proteins were extracted from dormant and germinating spores as described for BONCAT spore revival experiments. Extracts were incubated at 100ºC for 10 min with Laemmli sample buffer. Proteins were separated by SDS-PAGE 12.5% and electroblotted onto a polyvinylidene difluoride (PVDF) transfer membrane (Immobilon-P; Millipore). For Immunoblot analysis of GFP fusion proteins, membranes were blocked for 1 hr at room temperature (0.05% Tween-20, 5% skim milk in TBSx1). Blots were then incubated for 1 hr at room temperature with polyclonal rabbit anti-GFP antibodies (1:10,000 in 0.05% Tween-20, 5% skim milk in TBSx1). Next, membranes were incubated for 1 hour at room temperature with peroxidase conjugated goat anti-rabbit secondary antibody (Bio-Rad) (1:10,000 in 0.05% Tween-20, 5% skim milk in). EZ-ECL kit (Biological Industries, Beit Haemek, Israel) was used for final detection.

Spore resistance to lysozyme treatment

Spores were harvested from 5 ml DSM culture grown for 24 hrs and washed three times with cold water. The pelleted spores were resuspended in 1 ml of 0.05 M Tris-Cl buffer, pH 7.5, and the number of viable cells was determined. The spores were then incubated at 37°C for 60 min with lysozyme (50 µg/ml), and samples were plated to determine the number of survivors.

Plasmid construction

pLS50 (*tig-gfp-spc*), containing the 3’ region of *tig* fused to *gfp*, was constructed by amplifying the 3’ region of *tig* gene by PCR using primers 2534 and 2535 , which replaced the stop codon with a *Xho*I site. The PCR-amplified DNA was digested with *EcoR*I and *Xho*I and was cloned into the *EcoR*I and *Xho*I sites of pKL147 (*spc*) (Lemon and Grossman, 1998), which contains the *gfp* coding sequence.

pAR68 (*pupG-gfp-spc*), containing the 3' region of *pupG* fused to *gfp*, was constructed by amplifying the 3' region of *pupG* gene by PCR using primers 1929 and 1930 , which replaced the stop codon with a *Xho*I site. The PCR-amplified DNA was digested with *Mfe*I and *Xho*I and was cloned into the *EcoR*I and *Xho*I sites of pKL147 (*spc*) (Lemon and Grossman, 1998), containing the *gfp* coding sequence.

pAR71 (*malS-gfp-spc*), containing the 3' region of *malS* fused to *gfp*, was constructed by amplifying the 3' region of *malS* gene by PCR using primers 1323 and 1324 , which replaced the stop codon with a *Xho*I site. The PCR-amplified DNA was digested with *EcoR*I and *Xho*I and was cloned into the *EcoR*I and *Xho*I sites of pKL147 (*spc*) (Lemon and Grossman, 1998), containing the *gfp* coding sequence.

pLS100 (*amyE::rpmE-cat*), containing the *rpmE* gene (promoter and ORF) with flanking *amyE* sequences and *cat* gene, was constructed by amplifying the *rpmE* gene by PCR using primers 2556 and 2557 . The PCR-amplified DNA was digested with *BamH*I and *Hind*III and cloned into the *BamH*I and *Hind*III sites of pDG364 (*amyE::cat*) (Guerout-Fleury et al., 1996).

pLS101 (*amyE::tig-cat*), containing the *tig* gene (promoter and ORF) with flanking *amyE* sequences and a *cat* gene, was constructed by amplifying the *tig* gene by PCR using primers 2554 and 2555. The PCR-amplified DNA was cloned into the *BamH*I and *Hind*III sites of pDG364 (*amyE::cat*) (Guerout-Fleury et al., 1996) using Gibson Assembly cloning kit (New England Biolabs).

Primers used in this study

Name	**Target gene**	**Primer sequence**
881	*metE*	5'-CCTTGGAGGGCCAAGCGATGT-3'
882	*metE*	5'-ATTATGTCT TTTGCGCAG TCGGCCCCG CGGATA CAGGCTGCTAAGA-3'
883	*metE*	5'-CATTAATTT TGAGGGTTGCCAGCAGCCACAATC GGTTTCTTATTTAGCA-3'
884	*metE*	5'-GAGCTTGTCAACGCCGCTCA-3'
2526	*rpmE*	5'-CCCGGCTCTTACGCCACTTTATC-3'
2527	*rpmE*	5'-ATCACCTCAAATGGTTCGCTGGGTTTTGTATCCATC TCCTTCCGCCCTG -3'
2528	*rpmE*	5'-AAGTTC GCTAGATAGGGGTCCCGAGCTAATAGA TTTCTCAACAGGCAAGCAG -3'

2529	*rpmE*	5'-GGCCTTCTTTCGTTTTTTTTATGTTATT-3'
2536	*rpmE*	5'-ACATGTATTCACGAACGAAAATCGATGTATCCATC TCCTTCCGCCCTG -3'
2537	*rpmE*	5'-ATTTTAGAAAACAATAAACCCTTGCATAATAGATT TCTCAACAGGCAAGCAGCAG -3'
2556	*rpmE*	5'-AAACCCGGATCCATTGTATTTGCAAAAGAAGTAAA TCACTG-3'
2557	*rpmE*	5'-TAGTTTAAGCTTTTACTTAAGACCGTATTTTTTGTT AAAGCG-3'
2530	*tig*	5'-AAAGAAGGTTATGGCCATTATTTTAC-3'
2531	*tig*	5'-ATCACCTCAAATGGTTCGCTGGGTTTGTGTTTCCCT CCAAAAATCTATTCA -3'
2532	*tig*	5'-AAGTTCGCTAGATAGGGGTCCCGAGCAATAGTACT AATAAAACAGGGCGCG-3'
2533	*tig*	5'-GAATTCTTGATGAGGATGCTTACGT-3'
2534	*tig*	5'-TGGATCGAATTCATGAATTCGCAAAAG-3'
2535	*tig*	5'-TGGATCCTCGAGACGGTTTTCTACAAG-3'
2554	*tig*	5'-AAACCCGGATCCATCGCGCCATATAGTTGAAAGCG -3'
2555	*tig*	5'-AAACCCGGATCCTTAACGGTTTTCTACAAGAAAAT CAATTGC-3'
1323	*malS*	5'-TGGACTGAATTCCCGCCAGTTGAATATAACGGAGT TAC-3'
1324	*malS*	5'-TGGACTCTCGAGTATCGCGCGAATCGGTTTGTATA -3'
1929	*pupG*	5'-TGGACTCAATTGGCCAGATTTCCAGATATGTCTTC AGCC-3'
1930	*pupG*	5'-TGGACTCTCGAGTTCGTACTGAGCGACGATCGCTT TAAC-3'
2542	*malS*	5'- AGACAGACTCTGACAGGAGTCTG-3'
2543	*malS*	5'-ATCACCTCAAATGGTTCGCTGGGTTTCCTGCCTCTT CCTTTCTGAGCAT-3'
2544	*malS*	5'-AAGTTCGCTAGATAGGGGTCCCGAGCTTGCTTGTC CGGTGTTAAGAGGC-3'
2545	*malS*	5'-CTGTCCCGGTCTAATGCCGATTT-3'

2538	*maeA*	5'- ATTCGCTTCTGCTGATGGTCGTG-3'
2539	*maeA*	5'-ACATGTATTCACGAACGAAAATCGAGACCGATCATGATCTTTATGCGAC -3'
2540	*maeA*	5'-ATTTTAGAAAACAATAAACCCTTGCATTCTGTCCGAGGGAAAGCTTTTTG -3'
2541	*maeA*	5'- CAGAATCGCACTGACCACAAACG-3'
2546	*mleA*	5'-GTTAGGCATTCCGCTTCCTCTAGTAG-3'
2547	*mleA*	5'-GAACAACCTGCACCATTGCAAGATCAGTGATCCTCCCCTATTAGCCTAG-3'
2548	*mleA*	5'-TTGATCCTTTTTTTATAACAGGAATTCATGTAGCTAAACAGCACCTGTCGTAGC-3'
2549	*mleA*	5'-CAGAATGACCAGCAGCACAAAGGCATA-3'
2550	*ytsJ*	5'-CGCGTGCCGTCAAAAAGCGTAAATC-3'
2551	*ytsJ*	5'-ATTATGTCTTTTGCGCAGTCGGCCGTATACTGCTGCGTTGATTGCTGC-3'
2552	*ytsJ*	5'-CATTAATTTTGAGGGTTGCCAGGGCGCGCTGTTCTCAAGTTAAACTG-3'
2553	*ytsJ*	5'-CAGCGAAATTGTAATCGCCAAGGGA-3'
2656	*gerAA*	5'-TTCGAACGGTCCAGCATGTGAA-3'
2657	*gerAA*	5'-CTGAGCGAGGGAGCAGAATATGAAAGCGGAGGATACGAAGTGGC-3'
2658	*gerAA*	5'-GTTGACCAGTGCTCCCTGCAAATACAATGCTTGGGGCCGGACTT-3'
2659	*gerAA*	5'-CGTCATCCGGCCAGAGAGAAAAAT-3'

Supplemental References

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.

Driks, A., Roels, S., Beall, B., Moran, C.P., Jr., and Losick, R. (1994). Subcellular localization of proteins involved in the assembly of the spore coat of Bacillus subtilis. Genes Dev *8*, 234-244.

Elbaz, M., and Ben-Yehuda, S. (2010). The metabolic enzyme ManA reveals a link between cell wall integrity and chromosome morphology. PLoS Genet *6*, e1001119.

Guerout-Fleury, A.M., Frandsen, N., and Stragier, P. (1996). Plasmids for ectopic integration in *Bacillus subtilis*. Gene *180*, 57-61.

Guerout-Fleury, A.M., Shazand, K., Frandsen, N., and Stragier, P. (1995). Antibiotic-resistance cassettes for *Bacillus subtilis*. Gene *167*, 335-336.

Janssen, F.W., Lund, A.J., and Anderson, L.E. (1958). Colorimetric assay for dipicolinic acid in bacterial spores. Science *127*, 26-27.

Johnson, A.S., van Horck, S., and Lewis, P.J. (2004). Dynamic localization of membrane proteins in *Bacillus subtilis*. Microbiology *150*, 2815-2824.

Lemon, K.P., and Grossman, A.D. (1998). Localization of bacterial DNA polymerase: evidence for a factory model of replication. Science *282*, 1516-1519.

Peleg, Y., Rokem, J.S., Goldberg, I., and Pines, O. (1990). Inducible overexpression of the *FUM1* gene in *Saccharomyces cerevisiae*: localization of fumarase and efficient fumaric acid bioconversion to L-malic acid. Appl Environ Microbiol *56*, 2777-2783.

Youngman, P., Perkins, J.B., and Losick, R. (1984). Construction of a cloning site near one end of Tn917 into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene. Plasmid *12*, 1-9.