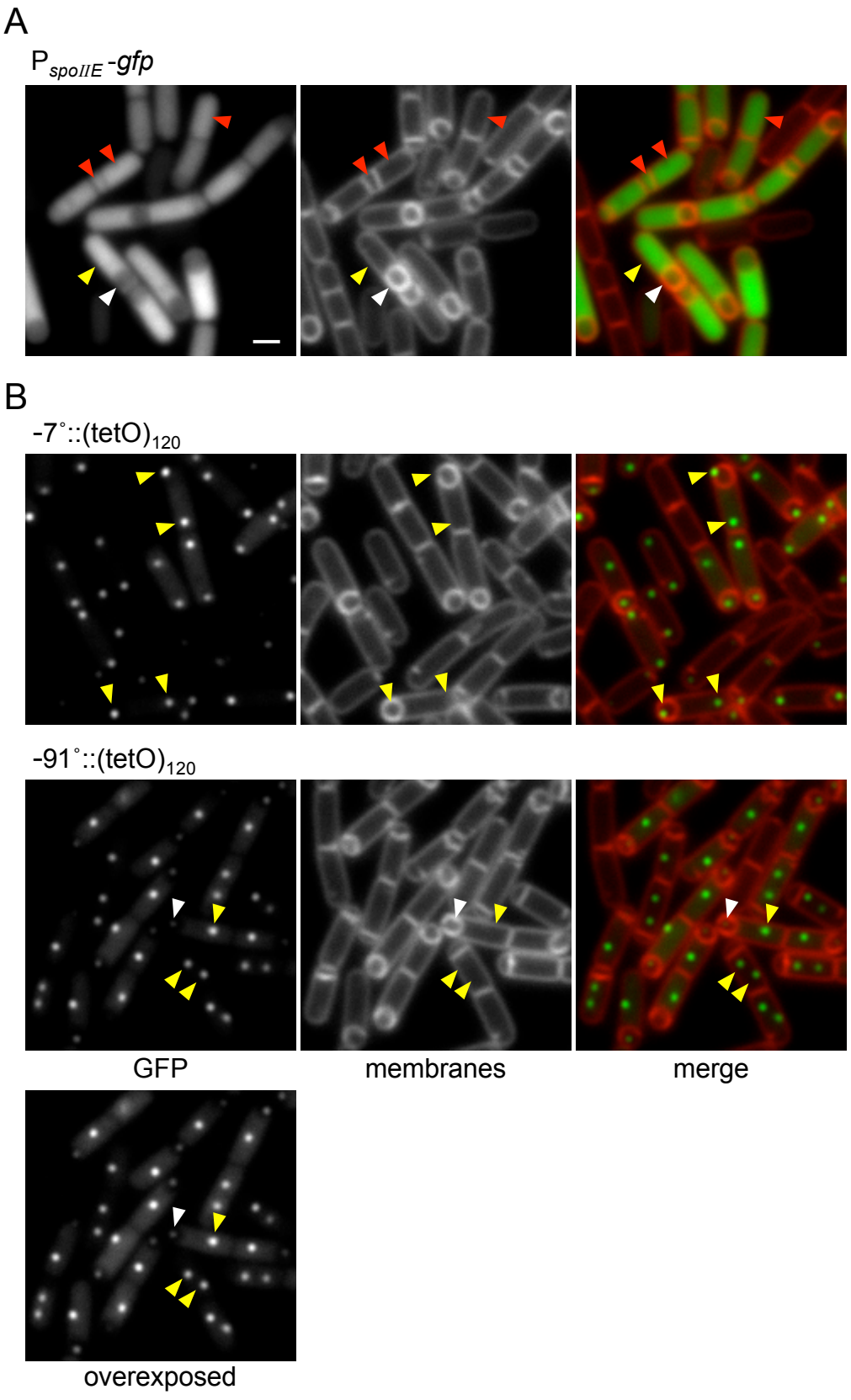


Supplemental Figure 1 (Marquis et al.)

Figure S1 TetR-GFP is stripped off the *tetO* array in sporulating cells that contain a wild-type copy of SpoIIIE. In these experiments *tetR-gfp* was fused to a promoter (P_{spoIIE}) that is activated prior to polar septation in the predivisional cell. (**A**) A strain (BDR2128) harboring a P_{spoIIE}-*gfp* fusion is shown early during sporulation. Transcription from the P_{spoIIE} promoter initiates prior to asymmetric division resulting in an even distribution of cytoplasmic GFP at the time of polar septation (red carets). After asymmetric division, transcription from this promoter persists in the mother cell but is down-regulated in the forespore compartment (compare yellow and white carets). (**B**) Fluorescence images of strains harboring the *tetR-gfp* fusion under the P_{spoIIE} promoter. In the upper panel the *tetO* array was placed at an origin-proximal site (-7˚) (strain BKM986) and in the lower panel it was placed at an original-distal site (-91˚) (strain BKM1245). TetR-GFP synthesized in the predivisional cell binds to the *tetO* arrays and generates two foci of equal intensity. Upon polar division, one of the -7˚ arrays is trapped in the forespore compartment. Although synthesis of TetR-GFP continues in the mother cell, the signal intensities of the foci in the mother cell and forespore remain similar (yellow carets). This suggests that TetR-GFP saturates the *tetO* binding sites prior to polar division. The -91˚ arrays are both present in the mother cell at the time of polar division and at this time the two foci have similar intensity (yellow carets). However, when the array on the forespore chromosome is translocated into the forespore the fluorescent signal is reduced (compare white and yellow carets). We interpret this to mean that the fusion protein is stripped off the DNA during tranlocation and then rebound by the sub-saturating levels of TetR-GFP present in the forespore. A rescaled (overexposed) image is shown to visualize the weak forespore foci. Scale bar, 1 μm.

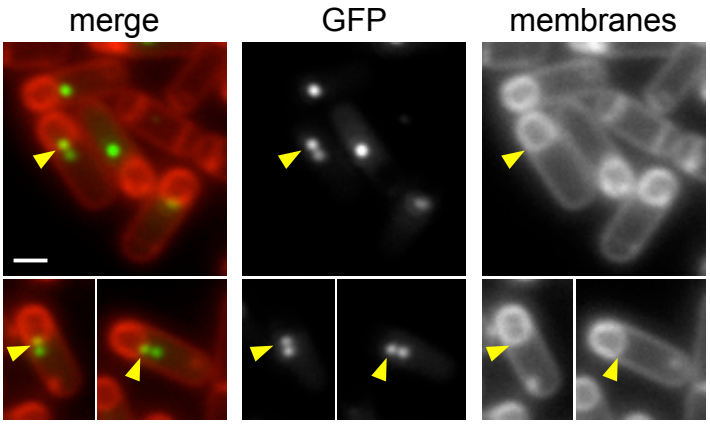


Figure S2 Resolution of the terminus regions of the mother cell and forespore chromosomes only occurs moments before translocation of the terminus into the forespore. The *tetO* operator array was inserted at 162˚ and the *tetR-gfp* fusion was placed under the control of a mother-cell-specific (σ^E-dependent) promoter. This strain (BKM938) also has a mutant SpoIIIE translocase that pumps the chromosome 2.5-fold slower than wild-type. At early time points prior to DNA translocation, most cells have a single focus in the mother cell. Two mother cell foci are only observed in cells in which the terminus region is about to be pumped into the forespore (yellow caret). Scale bar, 1 μm.

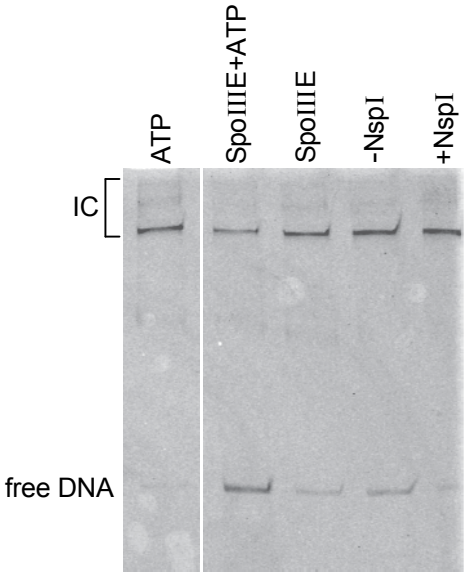
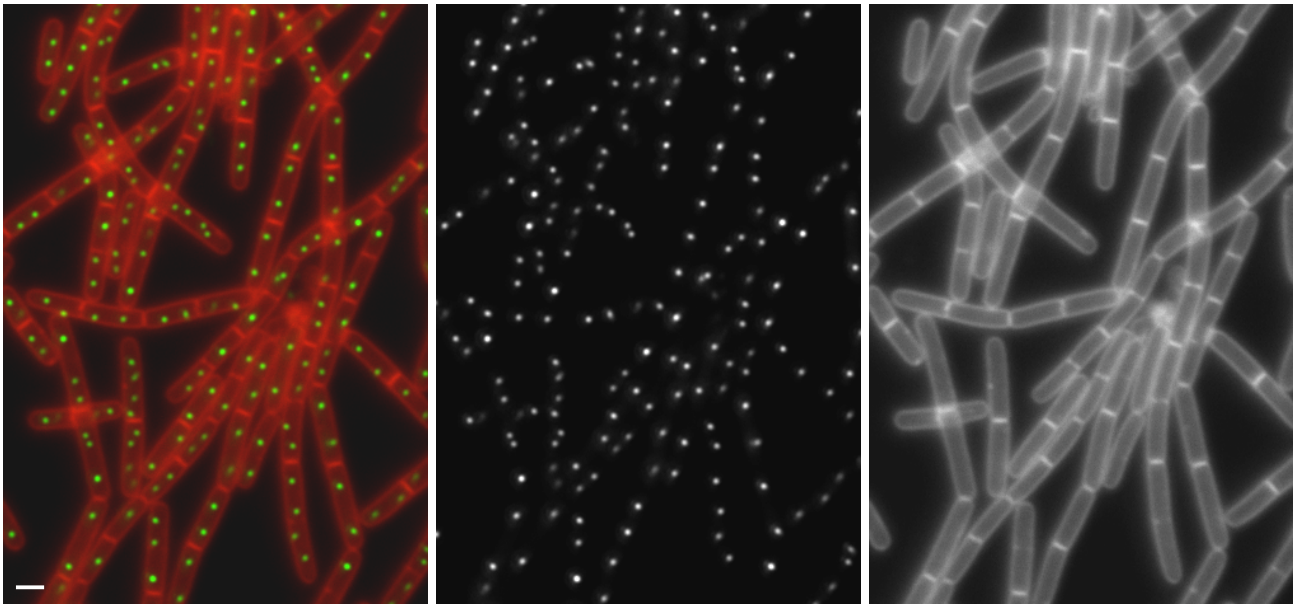


Figure S3 SpoIIIE-dependent displacement of a transcription initiation complex. *E. coli* RNA polymerase containing σ^{70} was incubated with a fluorescently-labeled DNA substrate containing the λ_{PR} promoter to generate an initiation complex (IC). The complexes were then incubated with 3 mM ATP, 120 nM SpoIIIE and 3mM ATP, or 120nM SpoIIIE and analyzed by gel mobility shift on a native polyacrylamide gel. The shifted complex is an initiation complex because a restriction endonuclease (NspI) was unable to cleave the DNA at a site within the promoter region.

A

inducer removed



B

one hour after inducer removed (replication road-block)

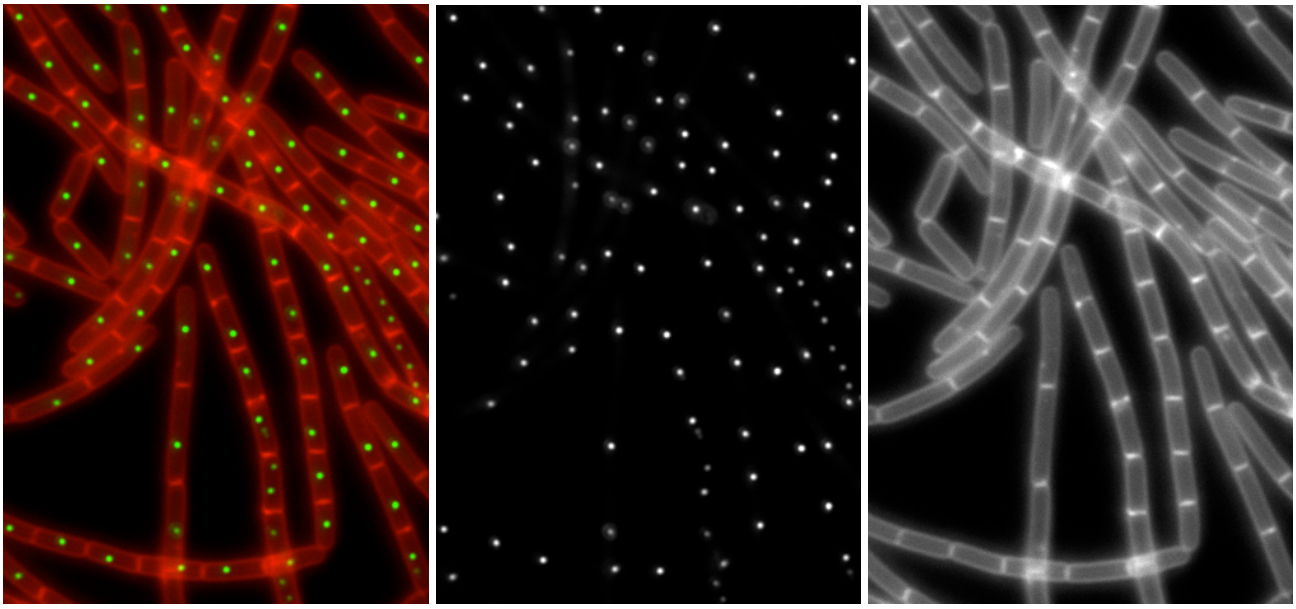


Figure S4 Respressor proteins bound to arrays of DNA binding sites cause a replication road-block. A strain (BKM898) harboring a *lacO* array (at 48˚) and *lacI-gfp* fused to a constitutive promoter was grown in the presence of IPTG to weaken the interaction between LacI-GFP and the array of operators. At mid-logarithmic growth the gratuitous inducer was removed by washing. The cells were visualized by fluorescence microscopy immediately after removal of the inducer and one hour after growth in its absence. (**A**) Immediately after the inducer was removed most cells have two foci indicating the presence of two copies of this region of the chromosome. (**B**) One hour later, virtually all cells have a single focus suggesting that LacI-GFP bound to the *lacO* array blocks progression of the replisome. Similar results were obtained with TetR-GFP and a *tetO* array (not shown). Scale bar, 1 μm.

Supplemental Figure 4 (Marquis et al.)

Table S1. *sporulation efficiency*

Strains	cfu/ml	spores/ml	spores/cfu	spores/wt spores
wild-type	6.9×10^8	5.6×10^8	81%	100%
spoIIIEΔ, ycgO::spoIIIE+	6.9×10^8	6.5×10^8	94%	116%
spoIIIEΔ, ycgO::spoIIIE^{D584A}	5.6×10^8	4.1×10^8	73%	73%
spoIIIEΔ, ycgO::spoIIIE^{D584A}, pelB::P_{spoIID}-tetR-gfp	5.3×10^8	4.2×10^8	79%	75%
spoIIIEΔ, ycgO::spoIIIE^{D584A}, pelB::P_{spoIID}-tetR-gfp (tet), 130°::$(tetO)_{240}$	4.1×10^8	3.0×10^8	73%	53%

Strains	cfu/ml	spores/ml	spores/cfu	spores/wt spores
wild-type	7.0×10^8	6.8×10^8	97%	100%
amyE::P_{spoIIE}-tetR-gfp	7.3×10^8	6.9×10^8	95%	101%
130°::$(tetO)_{240}$	4.5×10^8	3.7×10^8	82%	55%
amyE::P_{spoIIE}-tetR-gfp, 130°::$(tetO)_{240}$	6.2×10^8	5.8×10^8	94%	85%
amyE::P_{spoIIE}-tetR-gfp, -91°::$(tetO)_{120}$	6.6×10^8	6.2×10^8	94%	91%

Table S2. *Strains used in this study*

strain	genotype	reference
BDR1499	*rpoC-gfp (spec)*	a gift from M. Fujita
BDR1522	*rpoC-gfp (spec), spoIIGB::erm*	This work
BDR734	*amyE::P_{xylA}-gfp (cat)*	Rudner et al 2002
BDR1778	*amyE::$P_{spac(c)}$-gfp (spec)*	Fujita and Losick 2002
BKM986	*amyE::P_{spoIIE}-tetR-gfp (spec), yycR::$(tetO)_{120}$ (cat)*	This work
BKM1245	*amyE::P_{spoIIE}-tetR-gfp (spec), 269˚Ω$(tetO)_{120}$ (kan)*	This work
BKM938	*spoIIIE::neo, ycgO::spoIIIE(D584A)(cat), pelB::P_{spoIID}-tetR-gfp (tet), 162˚Ω$(tetO)_{120}$ (spec)*	This work
BKM898	*thrC::P_{pen}-lacIΔ11-gfp (erm), 48˚Ω$(lacO)_{120}$ (cat)*	This work
BKM1016	*spoIIIE::neo, ycgO::spoIIIE(D584A)(cat),pelB::P_{spoIID}-tetR-gfp (tet), 130˚Ω$(tetO)_{240}$ (spec)*	This work

Table S3. *plasmids used in this study*

plasmid	description
pKM193	*pelB::P_{spoIID}-tetR-gfp (tet) (amp)*
pKM219	*ykpAB* (130˚) - (*tetO*)$_{120}$ (*spec*) *(amp)*
pJP101	λ_{PR} promoter *(amp)*

Table S4. *oligonucleotide primers used in this study*

oligo	sequence
oDR78	gccGGATCCttatttgtatagttcatccatgcc
oDR188	gccAAGCTTacataaggaggaactactatgtctagattagataaaagtaaagtg
oDR458	gccAAGCTTagtgatagagaagacgaaccgtcc
oDR459	cggCTCGAgcattacagtttacgaaccgaacagg
oDR494	cgcGAATTCctctatcacttgaccaagcg
oDR495	cgcGGATCCgtaCTCGAGgtccgctgtatgctgtcg
oJP033	gataccggcgccgcaagggataaatatctaacaccgtgcgtg
oJP034	gataccgcggggatgacgccggacagcaccacagacc
oJP036	cggacaggtttgttagcccctacagct
oJP037	ggtagagctaaatgtctacaacgggattccgc

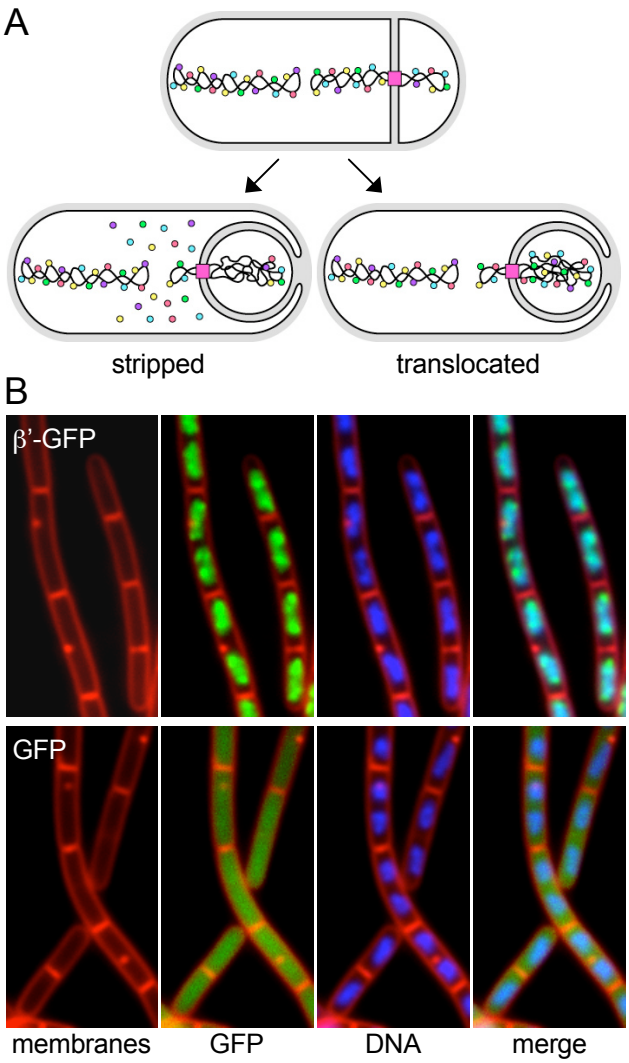


Figure 1 Two models for the fate of DNA binding proteins during chromosome translocation. (A) In the first model DNA associated proteins (colored circles) are stripped off the forespore chromosome during DNA transport. In the second model these proteins are translocated into the forespore along with the DNA. The SpoIIIE translocase (pink rectangle) is shown. The origins of replication are located near the cell poles and the termini are close to mid-cell. (B) RNA polymerase co-localizes with the chromosome (nucleoid) inside the cell. Fluorescent micrographs of vegetatively growing *B. subtilis* cells harboring a functional β'-GFP fusion (strain BDR1499). β'-GFP co-localizes with the DAPI-stained nucleoid. For comparison, a strain (BDR1778) producing free GFP localizes as a diffuse haze throughout the cytoplasm. A similar nucleoid-associated localization was observed for Hbsu-GFP (data not shown).

Figure 1 (Marquis et al.)

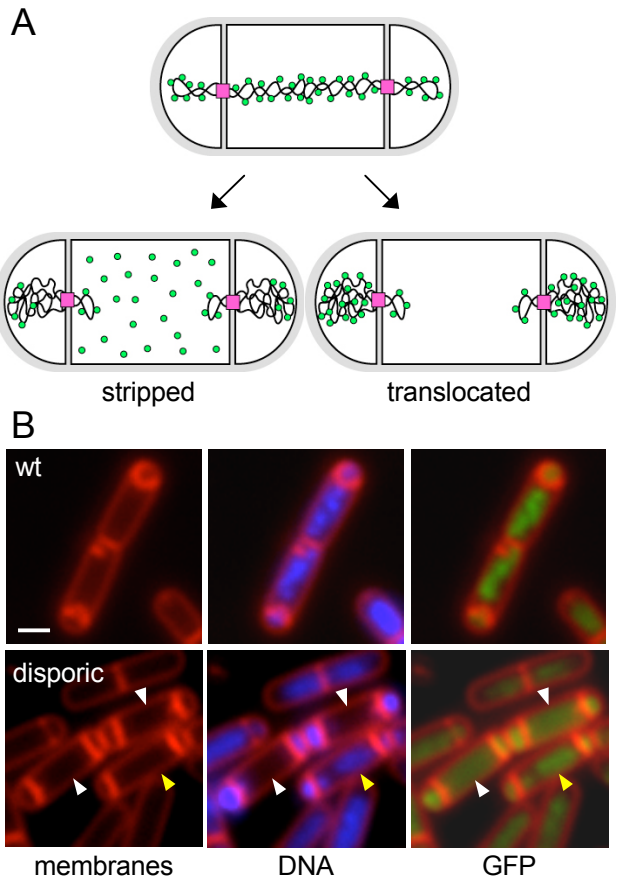


Figure 2 RNA polymerase is stripped off the DNA during chromosome translocation. (A) Schematic diagram of the experiment in B with the two possible outcomes. An abortively disporic mutant is shown with RNA polymerase (green balls) associated with the replicated chromosomes. If RNA polymerase is stripped off the DNA, the fluorescent fusion protein will be present as a diffuse haze in the mother cell cytoplasm. If RNA polymersase is translocated along with the DNA, the mother cell will be devoid of fluorescent signal. The SpoIIIE translocase (pink square) is shown. (B) β'-GFP is stripped off the chromosome during translocation into the forespore. Fluorescent micrographs of sporulating *B. subtilis* cells harboring a functional β'-GFP fusion. The upper panels show the co-localization of β'-GFP with the nucleoid in wild-type sporulating cells (strain BDR1499). The lower panels show β'-GFP in a disporic mutant (strain BDR1522). An abortively disporic sporangium that has not yet completed transport of the chromosomes into the forespore compartments is shown (yellow caret). In this cell β'-GFP co-localizes with the DNA that is still present in the mother cell compartment. Two abortively disporic sporangia that have finished DNA translocation are indicated (white carets). In these cells there is no DAPI signal in the mother cell and β'-GFP is visible as a diffuse haze in this compartment. The signal appears weaker because the protein is no longer concentrated on the DNA. Quantitation of fluorescent intensity revealed that 85-90% of the mother cell signal remains after DNA transport. Similar results were obtained with Hbsu-GFP and RacA-GFP (data not shown). Scale bar, 1 μm.

Figure 2 (Marquis et al.)

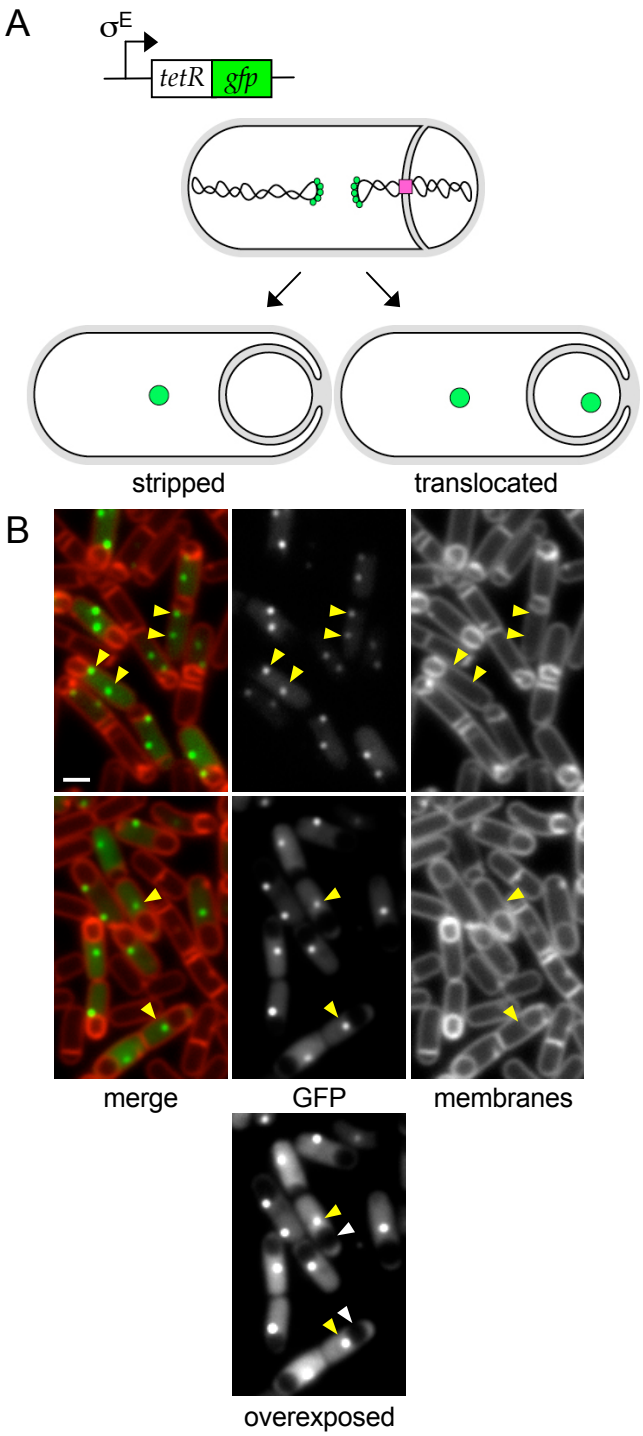


Figure 3 Transcriptional regulators are stripped off the DNA during translocation. (A) Schematic diagram of the experiment in B with the two possible outcomes. An array of *tetO* operators was inserted at a position near the teriminus (130˚) that is always present in the mother cell at the time of polar septation. In this strain, the *tetR-gfp* fusion was place under the control of a mother-cell-specific (σ^E-dependent) promoter. Finally, this strain has a mutant SpoIIIE translocase (pink square) that pumps the chromosome 2.5-fold slower than wild-type . Thus, at early time points after σ^E becomes active in the mother cell, two TetR-GFP foci should be visible in the mother cell. If TetR-GFP is stripped off the DNA one of these two foci will be missing when chromosome translocation is complete. If TetR-GFP is translocated with the chromosome, when DNA transport is complete, one TetR-GFP focus will be visible in the forespore and one in the mother cell. (B) TetR-GFP bound to the *tetO* array is stripped off the chromosome during translocation. Fluorescence micrographs of sporulating *B. subtilis* cells (strain BKM1016) at early (hour 2) and late (hour 3) time points. At hour 2, prior to the completion of DNA translocation two fluorescent foci are visible in the mother cell (yellow carets). At hour 3, when chromosome translocation is complete in most cells, a single focus is visible in the mother cell (yellow caret) and no detectable foci are seen in the forespore (white caret). No TetR-GFP foci are visible in the forespore when the micrograph is rescaled (overexposed) to reveal weak signals. Scale bar, 1 μm.

Figure 3 (Marquis et al.)

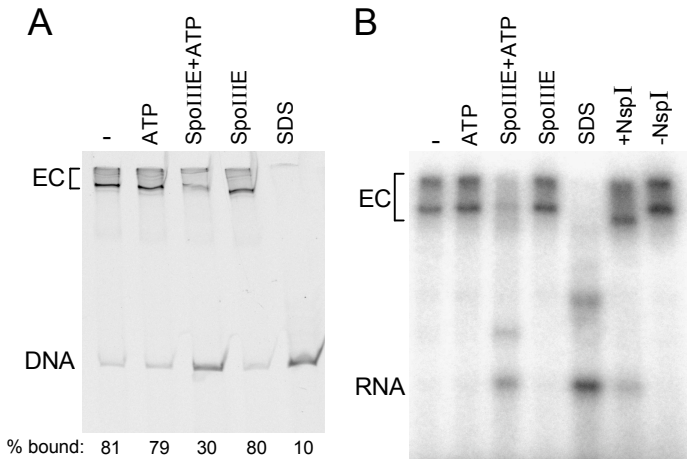


Figure 4 The soluble motor domain of SpoIIIE can displace a stalled transcription elongation complex in vitro. (**A**) *E. coli* RNA polymerase was incubated with a fluorescently-labeled DNA substrate containing the λ_{PR} promoter. The reaction contained 25 μM UTP, GTP, and ATP but no CTP to generate a stalled elongation complex (EC). The stalled complexes were then incubated with 3 mM ATP, 420 nM SpoIIIE and 3mM ATP, 420nM SpoIIIE or 5% SDS and analyzed by gel mobility shift on a native polyacrylamide gel. The fraction of RNA polymerase present in the elongation complex is indicated below the gel. (**B**) *E. coli* RNA polymerase was incubated with the λ_{PR} promoter and 2.5 μM UTP, GTP, ATP and 0.7 μM α^{32}P-GTP but no CTP to generate a stalled elongation complex with a short radiolabeled transcript. The stalled complex was incubated with 3 mM ATP, 200 nM SpoIIIE and 3mM ATP, 200nM SpoIIIE or 5% SDS and the reactions were analzyed on a native polyacrylamide gel followed by autoradiography. Dissassembly of the complex is evidenced by release of the labeled transcript. The stalled complex was confirmed by the ability of NspI to cut the DNA substrate (+NspI) within the promoter region resulting in a faster migrating complex.

Figure 4 (Marquis et al.)

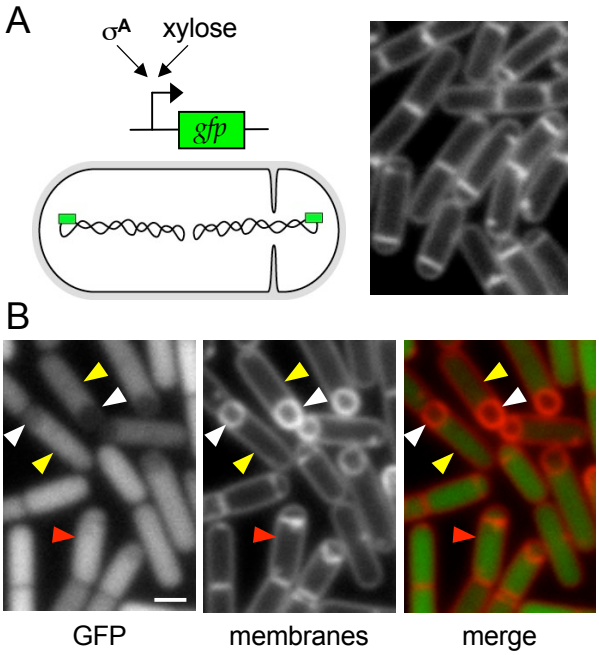


Figure 5 Vegetative gene expression is down-regulated in the forespore after chromosome translocation. As a result of the wire stripping activity of SpoIIIE, much of σ^A associated RNA polymerase will be stripped off the forespore chromosome during translocation. One prediction of this model is that σ^A-directed transcription will be reduced in the forespore compared to the mother cell. (A) The xylose-inducible σ^A promoter P_{xylA} was fused to *gfp* and placed at an original-proximal position of the chromosome (strain BDR734) and synchronous sporulation was induced in the absence of xylose. When ~40% of the sporulating cells had generated a polar septum (hour 1) xylose was added (20mM final concentration) to induce transcription from the P_{xylA} promoter. (B) GFP fluoresence was visualized 30 minutes later (hour 1.5). In sporulating cells in which DNA translocation had initiated or was complete, GFP fluorescence was reduced in the forespore (white caret) compared to the mother cell (yellow caret). In cells that had just assembled an asymmetric septum, the signal in both compartments was similar (red caret). Cell-to-cell variability in the intensity of the GFP signal is due to the differences in stages of sporulation at the time of xylose addition. Scale bar, 1 μm.

Figure 5 (Marquis et al.)