Variation of the folding and dynamics of the *Escherichia coli* chromosome with growth conditions

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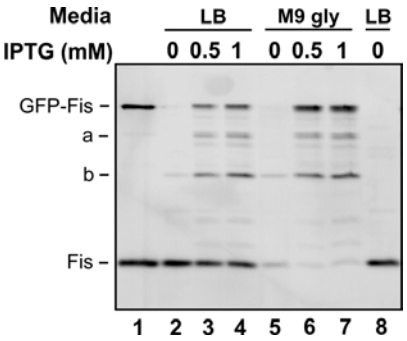
Supplementary Material

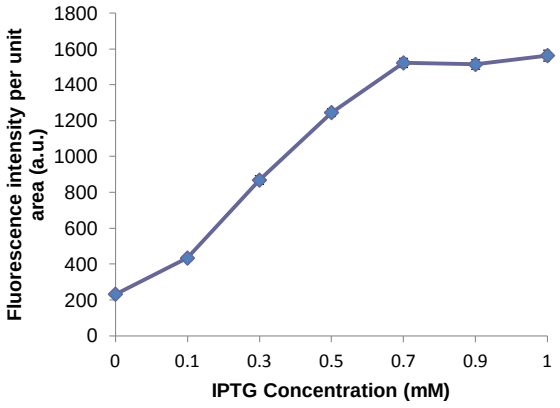
A



B

Strain	Media (mM IPTG)	GFP-Fis	Fis
FRAG1B pZE12-*GFP-fis*	LB (0)	-	22,450 ± 1,550
	LB (0.5)	1,450 ± 310	17,500 ± 700
	LB (1.0)	1,780 ± 550	17,950 ± 250
	M9 gly (0)	-	7,015 ± 1,685
	M9 gly (0.5)	5,960 ± 240	3,440 ± 1,070
	M9 gly (1)	5,210 ± 90	4,090 ± 570
MG1655 (*pyrE*$^{+}$ Δ*lacIZ*)	LB (0)	-	27,850
	M9 gly (0)	-	4,300

C



D

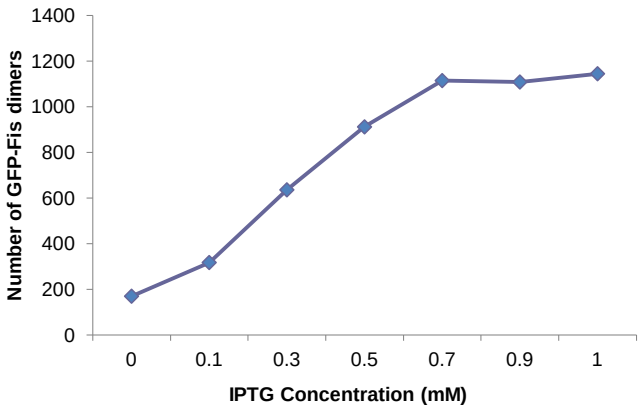


Fig. S1. GFP-Fis expression levels.

(**A**) Western blot probed with anti-Fis antibody. Lane 1 is purified GFP-Fis (8.5 ng) and Fis (20 ng). Lanes 2-7 are from FRAG1B pZE12-*GFP-fis* cells grown in LB (lanes 2-4) or M9 glycerol (lanes 5-7) with the indicated amount of IPTG. Lane 8 is MG1655 grown in LB. Band(s) a represent incompletely denatured full length GFP-Fis; band b is probably a GFP-Fis degradation product. (**B**) GFP-Fis or Fis dimers per cell from quantitative western blotting of cells growing in LB or M9 glycerol with the designated amounts of IPTG. Data for FRAG1B indicate average and range for two biological replicas and are compared to those from the wild-type *E. coli* strain MG1655.

Log phase cells in the indicated media were subcultured 1/1000 and grown at 30˚C to an OD_{600} = 0.1. Western blots on whole cell extracts were performed using the ECL2 system (Pierce-Thermo) after electroblotting onto PVDF paper and imaged on a Typhoon scanner. Amounts of GFP-Fis and Fis were determined from standard curves generated from known amounts of each purified protein loaded on the same gel and related to the colony forming units loaded.

(**C**) Average (N=50) fluorescence of GFP-Fis expressed inside cells as a function of IPTG concentration for rapid growth in LB. (**D**) Number of GFP-Fis dimers per cell for a range of IPTG concentration for growth in LB. Fluorescence intensity of a single GFP-Fis dimer in our setup was determined using the calibration method developed in our lab (Graham *et al*, 2011). Average total fluorescence intensity per cell was measured for about 40 cells at different IPTG levels from which the average number of GFP-Fis per cell was calculated.

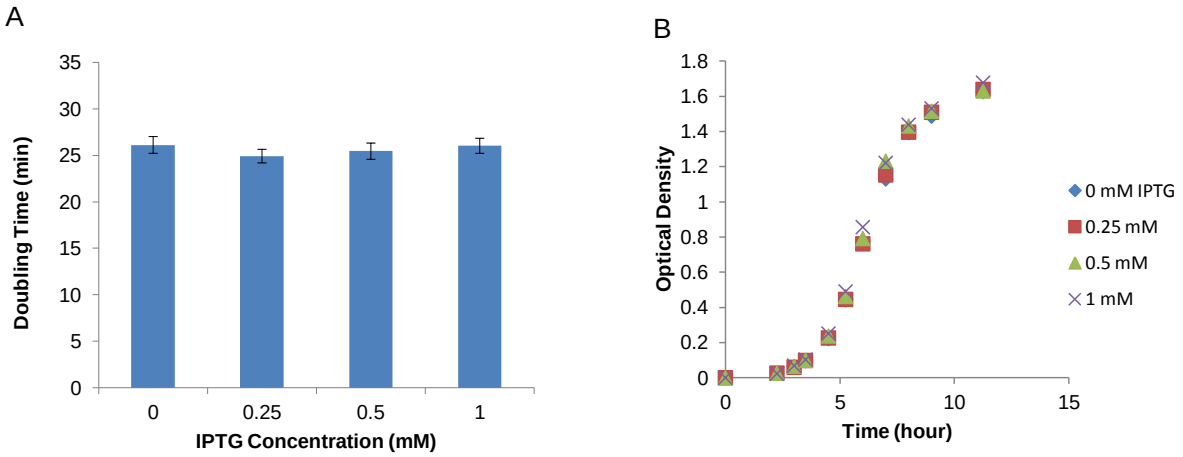


Fig. S2. Rapid growth in LB.

(**A**) Average (N=60) doubling times for cells growing under LB-agarose pad at 30°C and (**B**) Optical density, proportional to cell density in culture in rapid growth in LB at 30°C versus time, for a series of IPTG concentration showing no effect on growth.

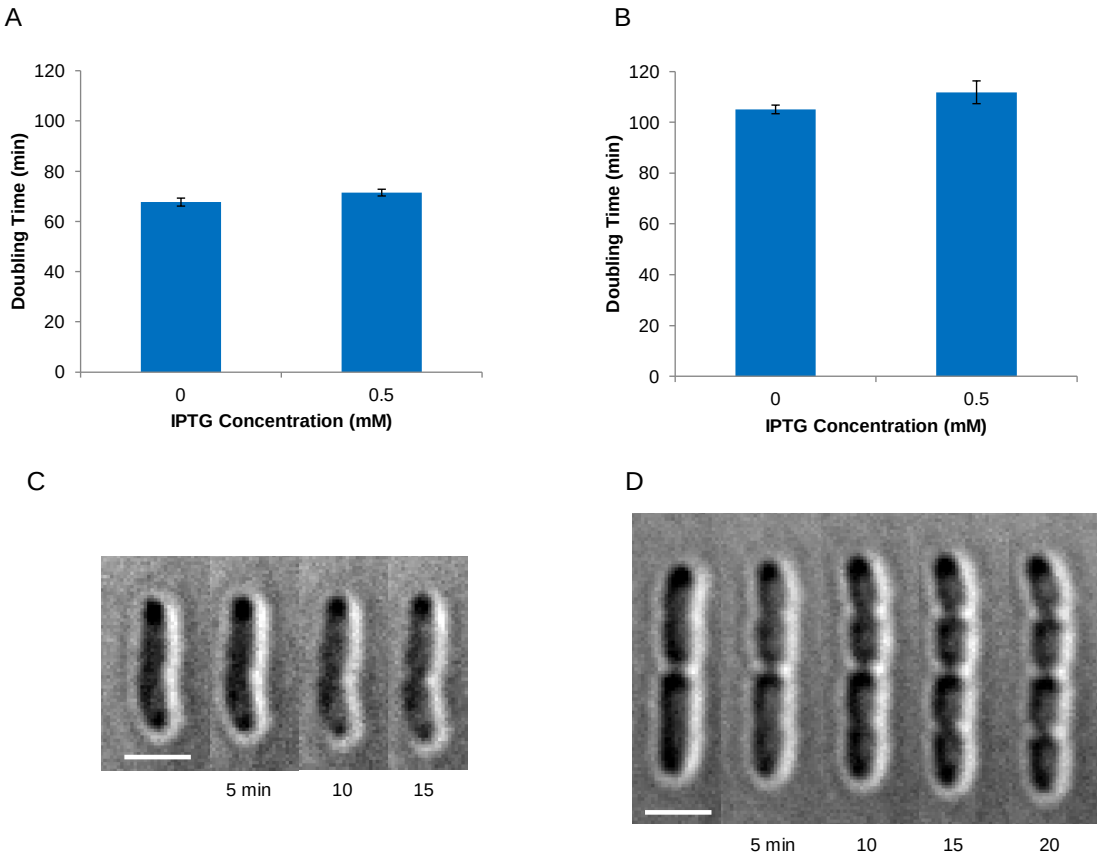


Fig. S3. Average doubling times during slow growth.
(A) Cells grown under M9 glycerol-agarose pad and (**B**) AB glucose-acetate agarose pad at 30°C, showing no significant effect of the expressed GFP-Fis levels on doubling times (N=50). Division times are measured from DIC images of the cells taken every 5 minutes. The reference "zero" time point for each cycle is defined to be the time at which the cells are clearly divided, which happens approximately 15-20 minutes after cells start to "pinch" (beginning of the septation process) for (**C**) M9 glycerol and (**D**) AB glucose-acetate. Bar is 2 µm.

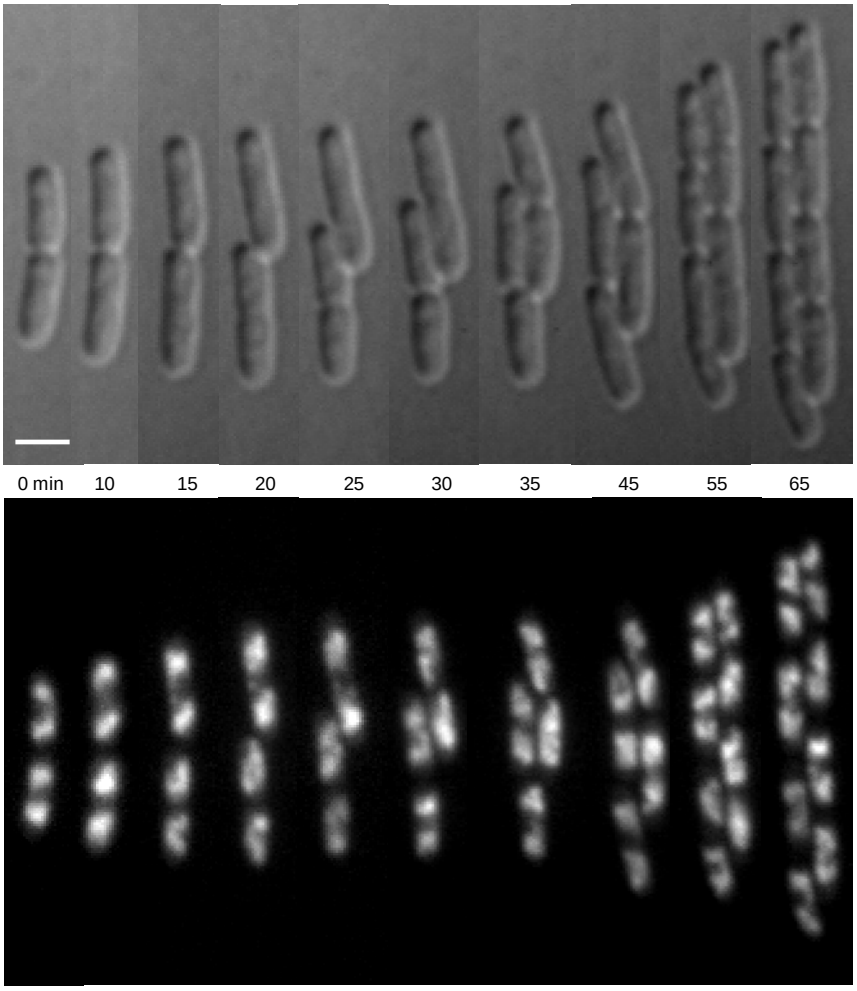


Fig. S4. Cells expressing *Anabaena* HU-GFP during rapid growth.
DIC and fluorescence images of cells growing and dividing (every 30 minutes at 30°C) under LB-agarose pad, expressing *Anabaena* HU-GFP. These cells are Frag1B strain carrying the IPTG inducible *pZS12hu-gfp* plasmid which was constructed as follows: using fusion PCR of the *hu* gene from *Anabaena* (a gift from Prof. Phoebe Rice) with the *gfp* gene we constructed *hu-gfp*. The internal primer used introduces a 5 amino acid linker (Gly-Gly-Gly-Gly-Ser) as used previously in Guet et al., 2008. The *hu-gfp* gene fusion was cloned into the KpnI and HindIII sites of a *pZS12* vector. Note that *Anaebena* HU is a homodimer unlike *E.coli* HU which is a heterodimer. Fluorescence images show the same general nucleoid patterns observed in cells expressing GFP-Fis, except for the haze around the nucleoids consistent with the weaker DNA-binding affinity of *Anabaena* HU relative to that of Fis. Bar is 2 μm.

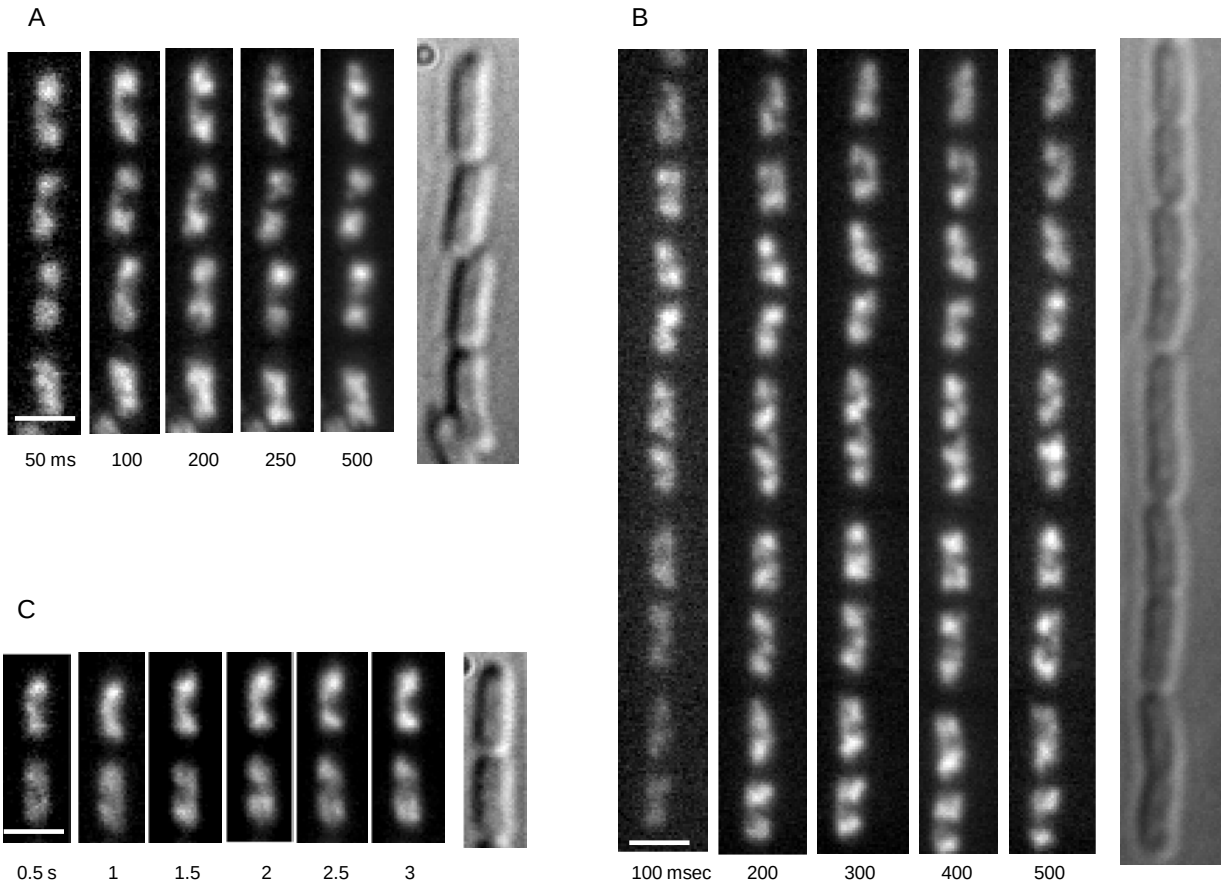


Fig. S5. Image acquisition with varied exposure times.
DIC images of the cells (expressing GFP-Fis) growing under LB-agarose pad, and fluorescence images of the nucleoids taken at different exposure times at (**A**) low (**B**) intermediate and (**C**) high laser power show no smearing for up to 3 sec exposure time. Time between fluorescence images is 30 sec. Bar is 2 µm.

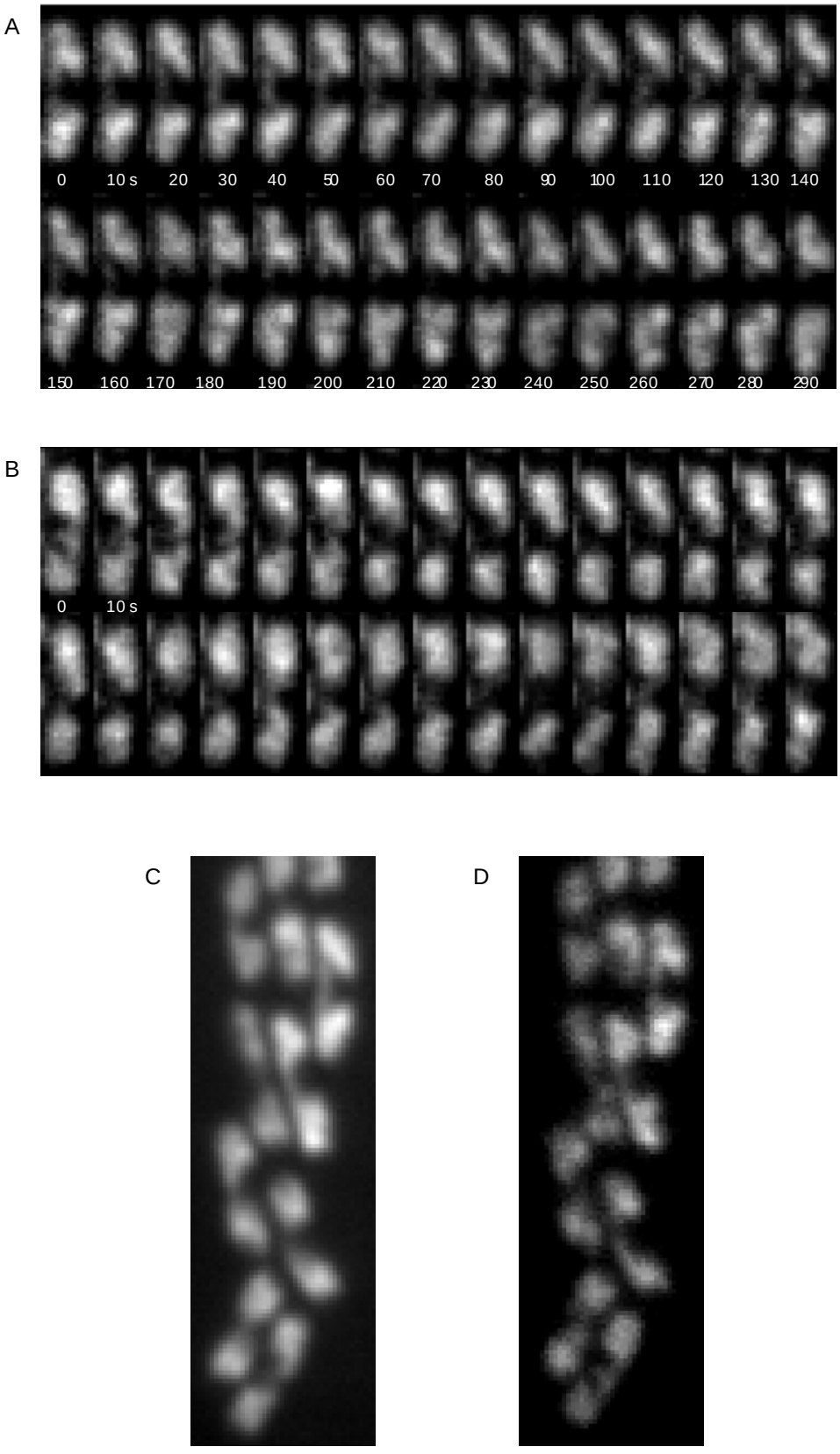


Fig. S6. Rapid sequence imaging during rapid growth.

Panels (**A**) and (**B**) show rapid sequence of images (10 sec between images) for two cells (growing in LB and expressing GFP-Fis) over a few minutes. (**C**) Average of images taken every 10 seconds over 2 minutes. (**D**) Image of the same nucleoids at time zero.

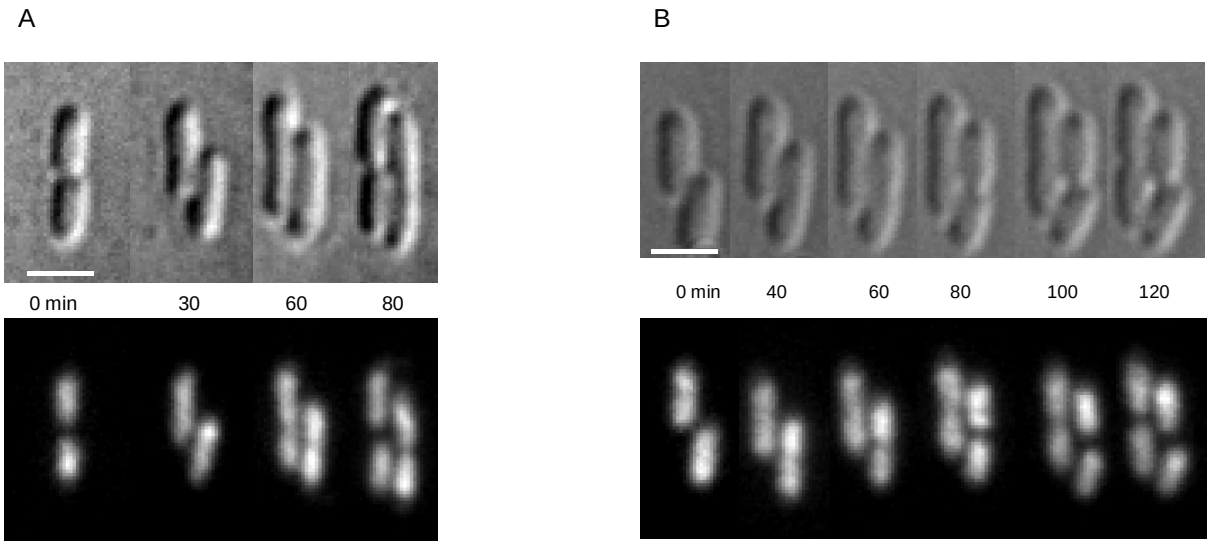


Fig. S7. Cells expressing *Anabaena* HU-GFP during slow growth.
DIC and fluorescence images of cells growing under (**A**) M9 glycerol-pad and (**B**) AB glucose-acetate agarose pad, expressing *Anabaena* HU-GFP. Fluorescence images show the same general nucleoid patterns observed in cells expressing GFP-Fis, except for the haze around the nucleoids consistent with the weaker DNA-binding affinity of *Anabaena* HU relative to that of Fis. Bar is 2 μm.

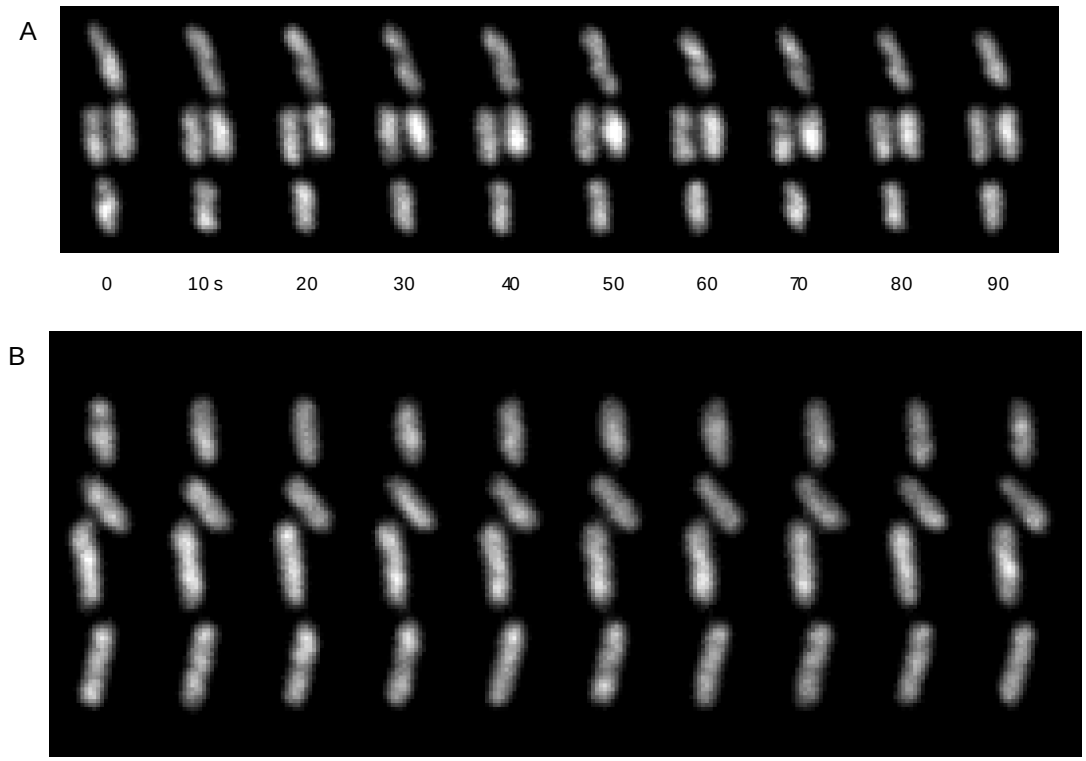


Fig. S8. Rapid sequence imaging during slow growth.
Montage of the rapid sequence images (10 sec between images) for cells (expressing GFP-Fis) grown in (**A)** M9 glycerol and (**B**) AB glucose-acetate.

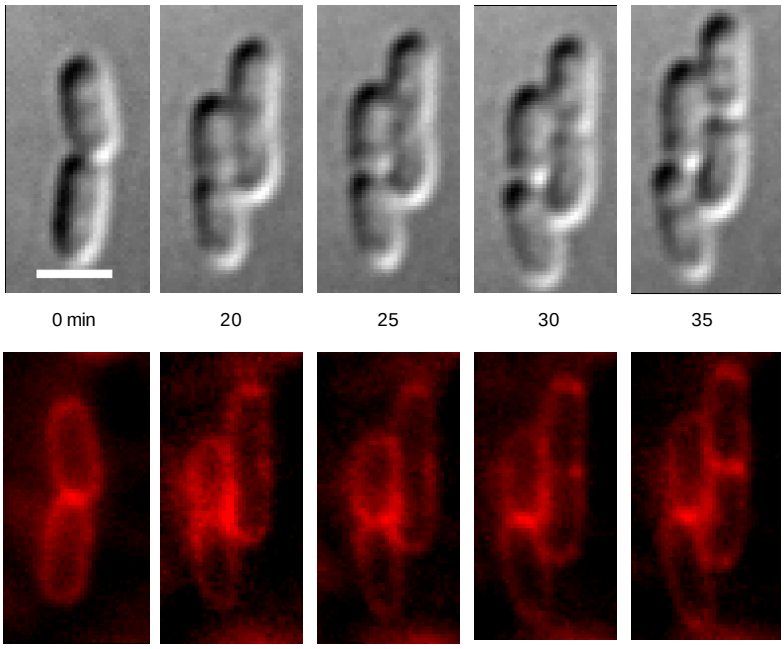


Fig. S9. Visualization of membrane at cell division.
DIC images of cells dividing under LB-agarose pad and fluorescence images of the membrane (using FM4-64 dye), showing the moment at which the septum that separates the parent cell into the two daughter cell compartments appears fully constructed and cells are clearly divided. This time point is defined as the reference “zero” time for measuring the doubling times of the microcolonies from DIC images of the cells taken every 2 minutes. Bar is 2 µm.

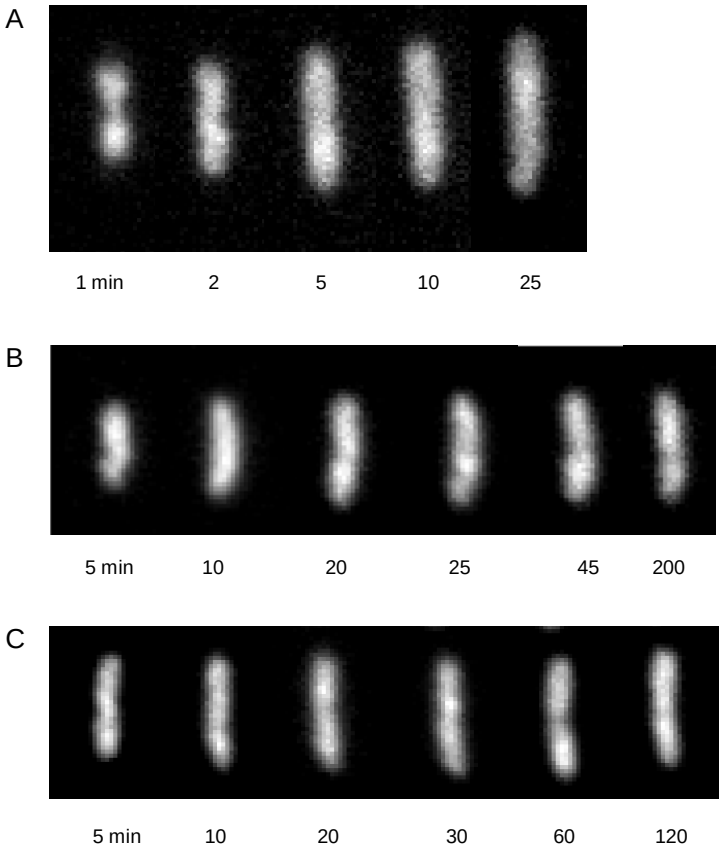


Fig. S10. Effect of rifampicin on nucleoid structure.
(**A**) Rapid nucleoid expansion in cells grown in LB, after treatment with rifampicin (100 µg/ml in the agarose pad). Less nucleoid decondensation during slow growth in (**B**) AB glucose-acetate and (**C**) M9 glycerol.

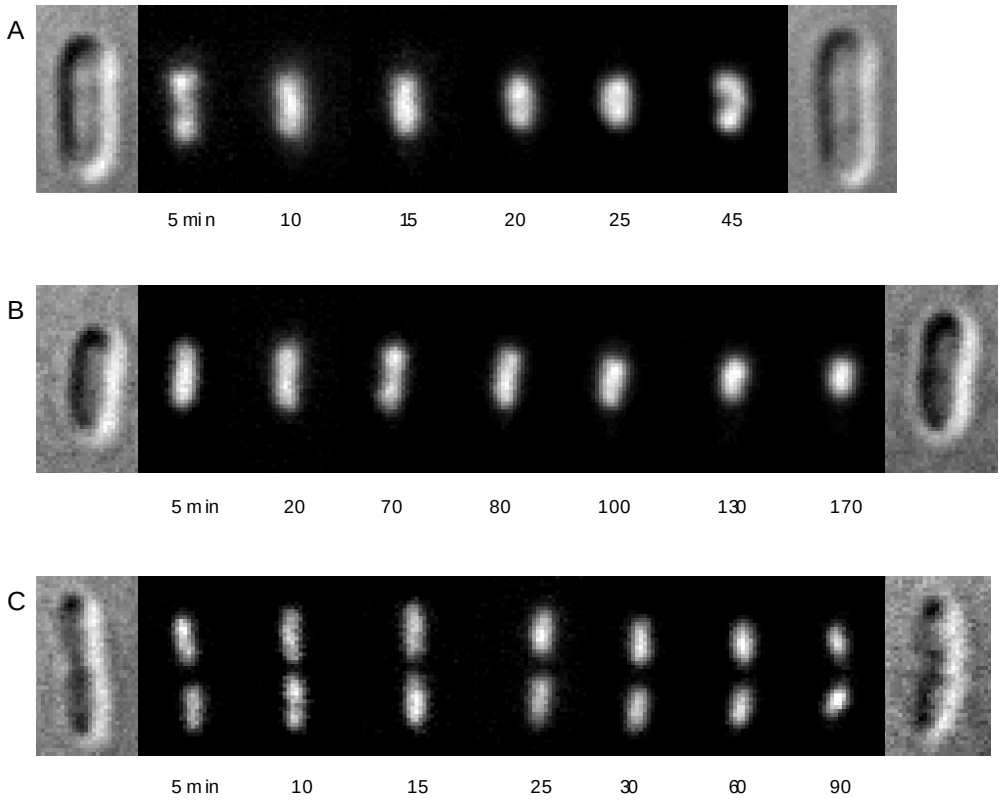


Fig. S11. Effect of chloramphenicol on nucleoid structure.
Nucleoid overcondensation after treatment with chloramphenicol (100 µg/ml in the agarose pad) during (**A**) rapid growth in LB, (**B**) slow growth in AB glucose-acetate and (**C**) slow growth in M9 glycerol.