Changes in the oligomerization potential of the division inhibitor UgtP coordinate *Bacillus subtilis* cell size with nutrient availability

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Supplemental Figures

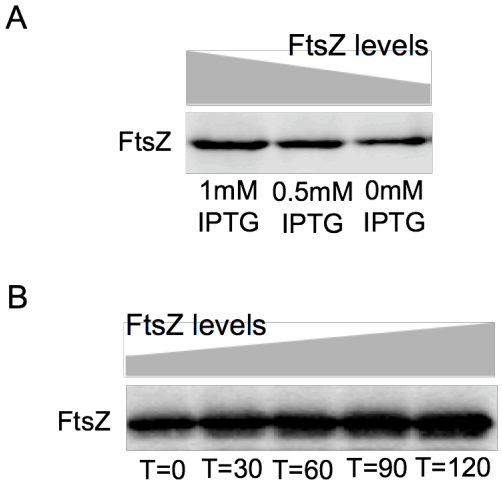

Figure S1. Quantitative immunoblots of FtsZ depletion and over-production. (A) The depletion of intracellular FtsZ levels in the strain PL2430 *amyE*::P_{xyl}-*yfp-ugtP ftsZ*::P_{spac}-*ftsZ* cultured with different amounts of the inducer IPTG. (B) The over-production of FtsZ over a 2 hours course of induction using the strain JC115 *amyE*::P_{spachy}-*yfp-ugtP thrC*::P_{xyl}-*ftsZ*.

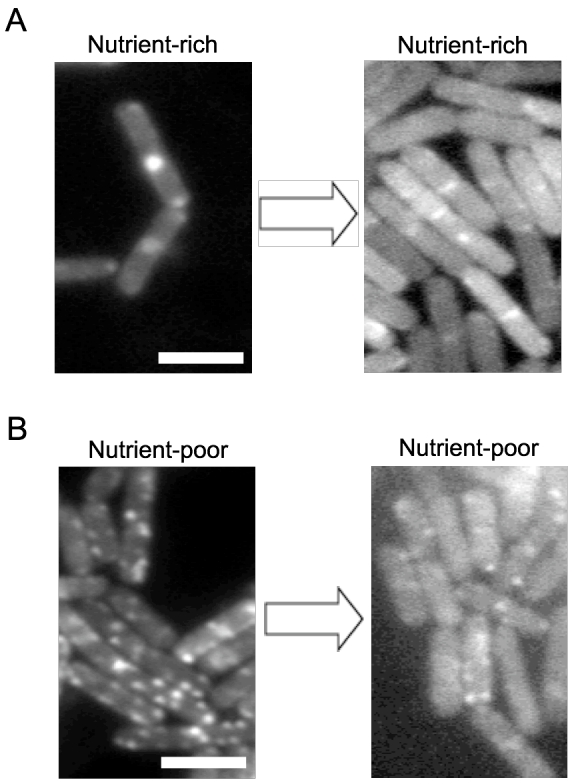

Figure S2. Controls of nutrient shift experiments. (A) Shifting PL2423 *amyE*::P^{xyl}-*yfp-ugtP* cells from nutrient-rich LB to nutrient-rich LB in the presence of 200 μg ml^{-1} chloramphenicol did not stimulate YFP-UgtP re-localization within 30 minutes. Bar = 3 μm. (B) Shifting PL2423 *amyE*::P^{xyl}-*yfp-ugtP* cells from nutrient-poor minimal sorbitol to nutrient-poor minimal sorbitol in the presence of 200 μg ml^{-1} chloramphenicol did not stimulate YFP-UgtP re-localization within 30 minutes. Bar = 3 μm. Cells were cultured at 30°C with a mass doubling time ~120 minutes.

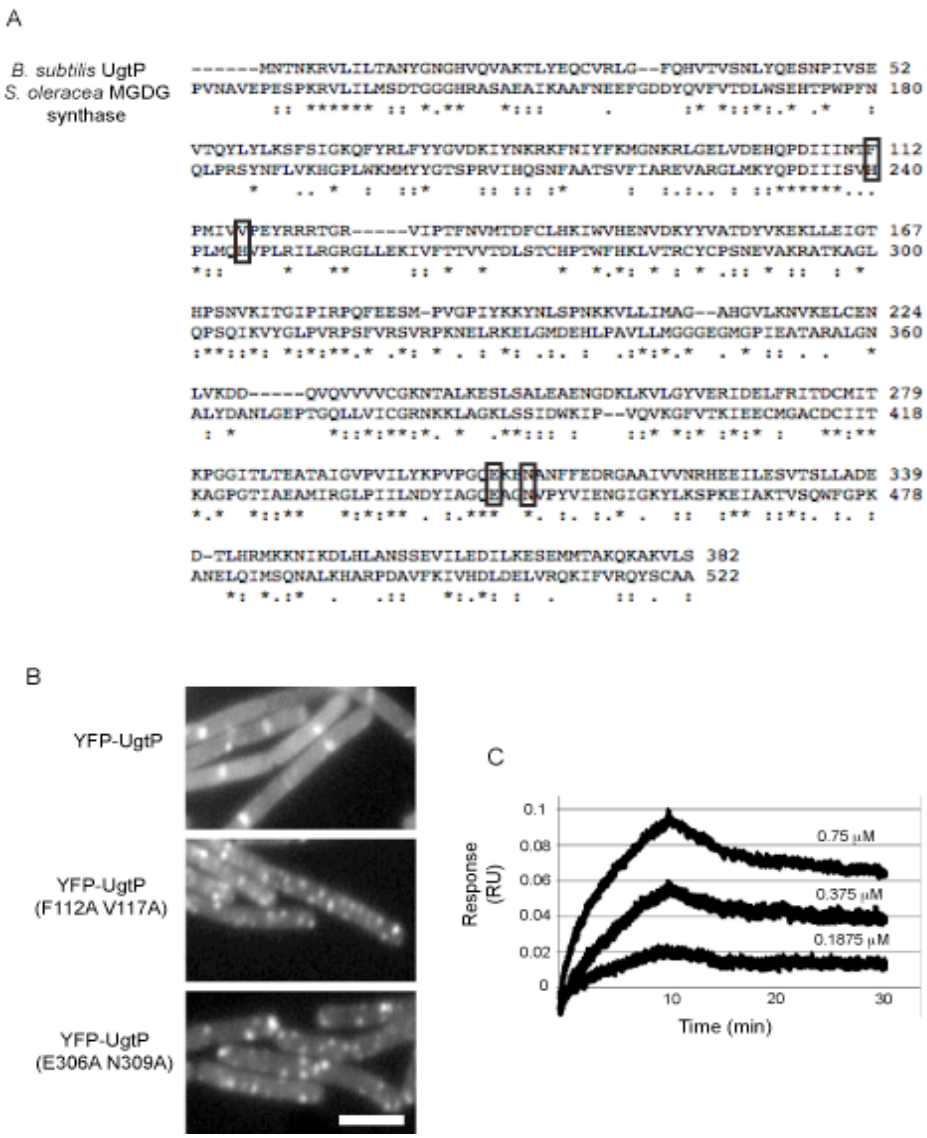

Figure S3. Sequence alignments, YFP-UgtP localization and representative Octet traces. (A) Sequence alignments of *B. subtilis* UgtP and *S. oleracea* MGDG synthase generated by ClustalW (Thompson *et al.*, 1994). Rectangles indicate residues predicted to be involved in nucleotide sugar UDP-glc binding: F112, V119, E306 and N309 in UgtP. (B) Localization of wild type YFP-UgtP, the putative nucleotide-binding mutant YFP-UgtP (F112A V117A), and the putative hexose-binding mutant YFP-UgtP (E306A N309A) in the presence of the wild type *ugtP* allele. (C) A representative overlay of the Octet traces of UgtP•UgtP interaction.

Figure S4. Quantitative immunoblots of FtsZ over-production in strains expressing the putative UDP-glc binding mutants of YFP-UgtP. Quantitative immunoblots of FtsZ indicating the over-production of FtsZ required for re-localization of the putative UDP-glc binding mutants of YFP-UgtP. YFP-UgtP (F112A V117A) is the putative nucleotide-binding mutant while YFP-UgtP (E306A and N309A) is the putative hexose-binding mutant. JC215 *amyE*::P_{xyl}*-yfp-ugtP (F112A V117A) thrC*::P_{spachy}*-ftsZ* and JC291 *amyE*::P_{xyl}*-yfp-ugtP (E306A N309A) thrC*::P_{spachy}*-ftsZ* cells were used.

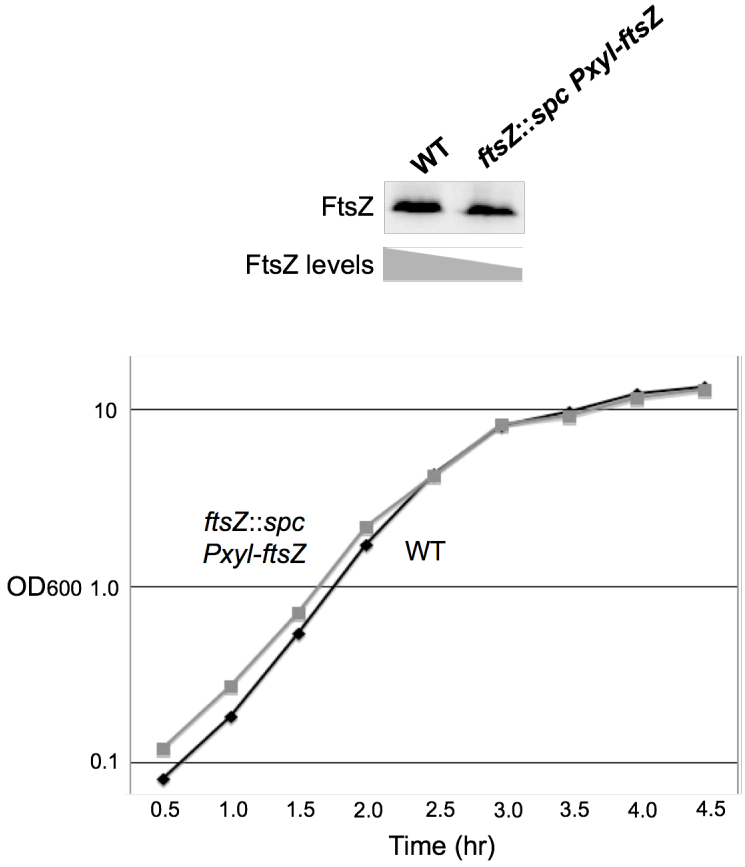

Figure S5. Growth curves of wild type cells and partially FtsZ-depleted cells. Top: Quantitative immunoblots indicate that PL2084 *ftsZ*::*spc thrC*::P_{xyl}-*ftsZ* cells cultured in LB supplemented with 0.1% xylose display ~15% reduction in intracellular FtsZ levels compared to wild type cells cultured in LB. Bottom: PL2084 *ftsZ*::*spc thrC*::P_{xyl}-*ftsZ* cells cultured in LB supplemented with 0.1% xylose display similar growth curve (gray) compared to that of wild type cells cultured in LB (black).

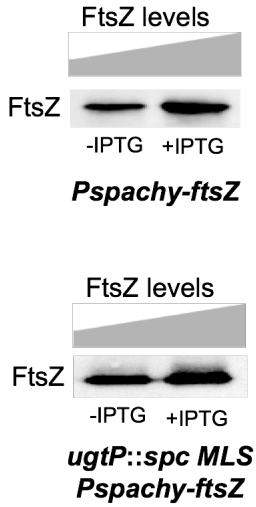

Figure S6. Quantitative immunoblots of partial FtsZ over-production. Quantitative immunoblots indicate the levels of FtsZ over-production (~13%) using the *amyE*::P_{spachy}-*ftsZ* construct in both wild type (top) and *ugtP*::*spc MLS* (bottom) backgrounds

Supplemental Tables

Table S1. Strain table

Strain	Genotype	Reference
JH642	*B. subtilis trpC2 pheA1*	(Perego *et al.*, 1988)
PL2292 (BW503)	JH642 *pgcA*::*Tn10 cat*::*spc amyE*::P_{xyl}-*yfp-ugtP cat*	(Weart *et al.*, 2007)
PL2430 (AL221)	JH642 *ezrA*::*ezrA-cfp cat amyE*::P_{xyl}-*yfp-ugtP cat*::*spc ftsZ*::P_{spac}-*ftsZ phleo*	(Weart *et al.*, 2007)
JC115	JH642 *amyE*::P_{spachy}-*yfp-ugtP thrC*::P_{xyl}-*ftsZ*	This work
JC313	JH642 *amyE*::P_{xyl}-*yfp-ugtP thrC*::P_{spachy}-*gtaB-myc gtaB*::*spc*	This work
JC330	JH642 *amyE*::P_{xyl}-*yfp-ugtP thrC*::P_{spachy}-*ywqF*	This work
PL2423 (AL198)	JH642 *amyE*::P_{xyl}-*yfp-ugtP cat*	(Weart *et al.*, 2007)
PL2295 (BW507)	JH642 *ugtP*::*cat*::*spc erm amyE*::P_{xyl}-*yfp-ugtP cat*	(Weart *et al.*, 2007)
JC41	JH642 *amyE*::P_{xyl}-*yfp-ugtP(F112A V117A) cat*	This work
JC47	JH642 *ugtP*::*cat*::*spc erm amyE*::P_{xyl}-*yfp-ugtP (F112A V117A) cat*	This work
JC275	JH642 *amyE*::P_{xyl}-*yfp-ugtP(E306A N309A) cat*	This work
JC283	JH642 *ugtP*::*cat*::*spc erm amyE*::P_{xyl}-*yfp-ugtP (E306A N309A) cat*	This work
JC215	JH642 *amyE*::P_{xyl}-*yfp-ugtP (F112A V117A) cat thrC*::P_{spachy}-*ftsZ*	This work
JC291	JH642 *amyE*::P_{xyl}-*yfp-ugtP (E306A N309A) cat thrC*::P_{spachy}-*ftsZ*	This work
PL2084 (BW121)	JH642 *ftsZ*::*spc xylA*::*tet thrC*::P_{xyl}-*ftsZ*	This work
PL950	JH642 *amyE*::P_{spachy}-*ftsZ cat*	(Weart & Levin, 2003)
JC438	JH642 *amyE*::P_{spachy}-*ftsZ cat ugtP*::*cat cat*::*spc erm*	This work

Supplemental Experimental Procedures

Plasmid and strain construction

Standard techniques were used for genetic manipulations. Ampicillin was used at 100 μg ml^{-1}, chloramphenicol at 5 μg ml^{-1}, phleomycin at 2 μg ml^{-1}, and spectinomycin at 100 μg ml^{-1}. MLS resistance was selected for using erythromycin at 0.5 μg ml^{-1} and lincomycin at 12.5 μg ml^{-1}.

Quantitative immunoblotting

Mid-exponential phase cells were harvested and resuspended in 50 mM Tris pH 8.0, 1 mM EDTA, 2 mg ml^{-1} lysozyme and 1 mM AEBSF. Cells were incubated at 37°C for 15 minutes, chilled at 4°C for 15 minutes, and then lysed by the addition of sodium dodecyl sulfate (SDS). Cell lysates were normalized to OD_{600} at gel loading and subjected to SDS-polyacrylamide gel electrophoresis. FtsZ was probed using affinity purified polyclonal rabbit anti-FtsZ antibodies. Primary antibodies were used in conjunction with a goat anti-rabbit antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch). Immunoblots were quantitated via chemiluminescence using a LAS1000plus imager in conjunction with ImageGauge software v3.41 (Fuji Film).

Supplemental References

Perego, M., G. B. Spiegelman & J. A. Hoch, (1988) Structure of the gene for the transition state regulator, abrB: regulator synthesis is controlled by the spo0A sporulation gene in *Bacillus subtilis*. *Mol Microbiol* **2**: 689-699.

Thompson, J. D., D. G. Higgins & T. J. Gibson, (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673-4680.

Weart, R. B., A. H. Lee, A. C. Chien, D. P. Haeusser, N. S. Hill & P. A. Levin, (2007) A metabolic sensor governing cell size in bacteria. *Cell* **130**: 335-347.

Weart, R. B. & P. A. Levin, (2003) Growth rate-dependent regulation of medial FtsZ ring formation. *J Bacteriol* **185**: 2826-2834.