Dimerization or oligomerization of the actin-like FtsA protein enhances the integrity of the cytokinetic Z ring

Daisuke Shiomi and William Margolin*

Microbiology and Molecular Genetics, University of Texas Medical School,

6431 Fannin Houston, TX 77030

*Corresponding author.

Mailing address: Microbiology and Molecular Genetics, University of Texas Medical School, 6431 Fannin Houston, TX 77030

Phone: 713-500-5452

FAX: 713-500-5499

E-mail: William.Margolin@uth.tmc.edu

Supplementary Materials

SUPPLEMENTARY TABLE AND LEGENDS TO SUPPLEMENTARY FIGURES

Table S1. Oligonucleotides used in this study.

Oligonucleotide number and name		Sequence
822.	ftsA Xbal FP	tat ata tct aga atg atc aag gcg acg gac aga
823.	ftsA Stop PstI RP	tat ata ctg cag tta aaa ctc ttt tcg cag cca
826.	ftsA SacI FP	tat ata gag ctc atg atc aag gcg acg gac aga
827.	ftsA non stop Xbal RP	tat ata tct aga aaa ctc ttt tcg cag cca act
906.	ftsA-M71A-f2	cat tga cca ggc aga att gGC Cgc aga ttg tca gat ctc
907.	ftsA-M71A-r2	gag atc tga caa tct gcG GCc aat tct gcc tgg tca atg
951.	ftsA-R286W-f	ggt cgt ccg cca TGG agt ctg caa cgt c
952.	ftsA-R286W-r	gac gtt gca gac tCC Atg gcg gac gac c
1100.	ftsA-R300E-f	gtg atc gag ccg GAG tat acc gag ctg
1101.	ftsA-R300E-r	cag ctc ggt ata CTC cgg ctc gat cac
989.	ftsA-d126-133-f	gtg cgc gat gag cat gag tat gcg att gac
990.	ftsA-d126-133-r	gtc aat cgc ata ctc atg ctc atc gcg cac
1003.	ftsA-I143L-f	cta tca gga agg gCT Caa gaa tcc ggt agg ac
1004.	ftsA-I143L-r	gtc cta ccg gat tct tGA Gcc ctt cct gat ag
1029.	ftsA-D66X-f	caa cgc gcc att NNN cag gca gaa ttg
1030.	ftsA-D66X-r	caa ttc tgc ctg NNN aat ggc gcg ttg
1031.	ftsA-Q67X-f	gcc att gac NNN gca gaa ttg
1032.	ftsA-Q67X-r	caa ttc tgc NNN gtc aat ggc
1033.	ftsA-E69X-f	gac cag gca NNN ttg atg gca gat tg
1034.	ftsA-E69X-r	caa tct gcc atc aaN NNt gcc tgg tc
1035.	ftsA-M71X-f	cat tga cca ggc aga att gNN Ngc aga ttg tca gat ctc
1036.	ftsA-M71X-r	gag atc tga caa tct gcN NNc aat tct gcc tgg tca atg
918.	FLAG-f (NdeI)	gga att cca tat gga cta caa gga cga cga tga caa a
528.	ftsAC-term (XhoI)	ttt ctc gag tta aaa ctc ttt tcg c
696.	pDSW-reverse	caa att ctg ttt tat cag ac
971.	zipA-f (NdeI)	cgc cat atg atg cag gat ttg cgt ctg
972.	zipA-r (BamHI)	gcg gat cct cag gcg ttg gcg tct ttg
973.	zapA-f (NdeI)	cgc cat atg tct gca caa ccc gtc gat atc c
974.	zapA-r (BamHI)	gcg gat cct cat tca aag ttt tgg tta g
975.	gfp-f (NsiI)	cca atg cat aaa gga gaa gaa ctt ttc ac
977.	ftsN-r (XhoI)	ccc tcg agt caa ccc ccg gcg gcg
965.	ftsZ-f (PstI)	gcc tgc agg gtt tga acc aat gga act tac c
967.	ftsZ-r (XhoI)	ccc tcg agt taa tca gct tgc tta cgc agg aat gc
650.	ftsA1PKT25	aaa act gca ggg atg atc aag gcg acg
968.	ftsA-f (PstI)	gcc ctg cag cat caa ggc gac gga cag aaa act gg
876.	ftsA-r9 (EcoRI)	cgg aat tct taa aac tct ttt cgc agc c
646.	ftsZ1-pKT25	aaa act gca gcg atg ttt gaa cca atg
647.	ftsZ2-pKT25	cgc gga tcc tta atc agc ttg att a

Supplementary Figure legends

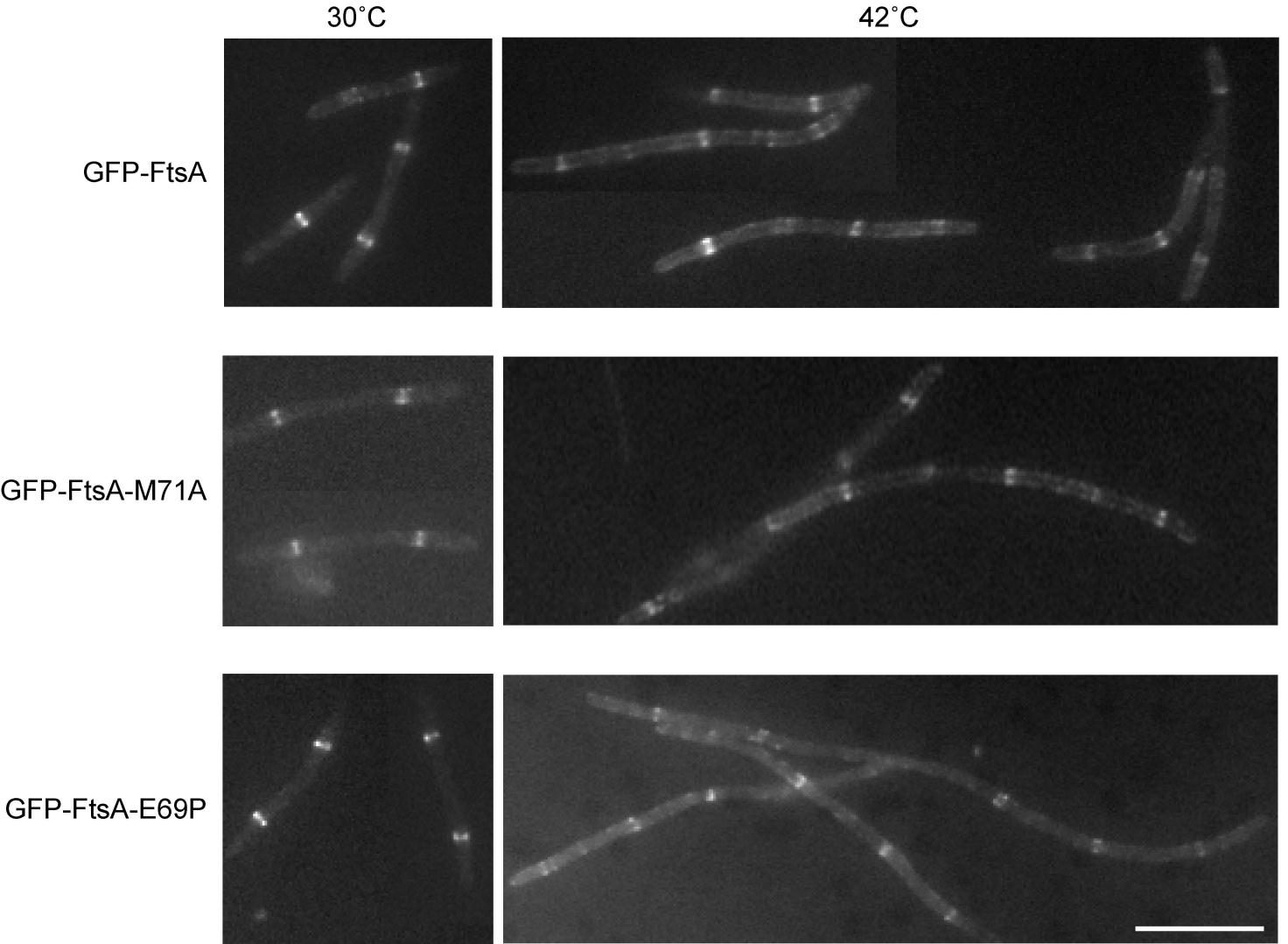
Fig. S1. Localization of GFP-FtsAs. WM1115 (*ftsA12*) cells carrying pWM3196 (GFP-FtsA), pWM3197 (GFP-FtsA-M71A) or pWM3200 (GFP-FtsA-E69P) were incubated at 30˚C for 2 h in the presence of 0.75 μM sodium salicylate (left panels), then shifted to 42˚C for 30 min (right panels). Scale bar is 10 μm.

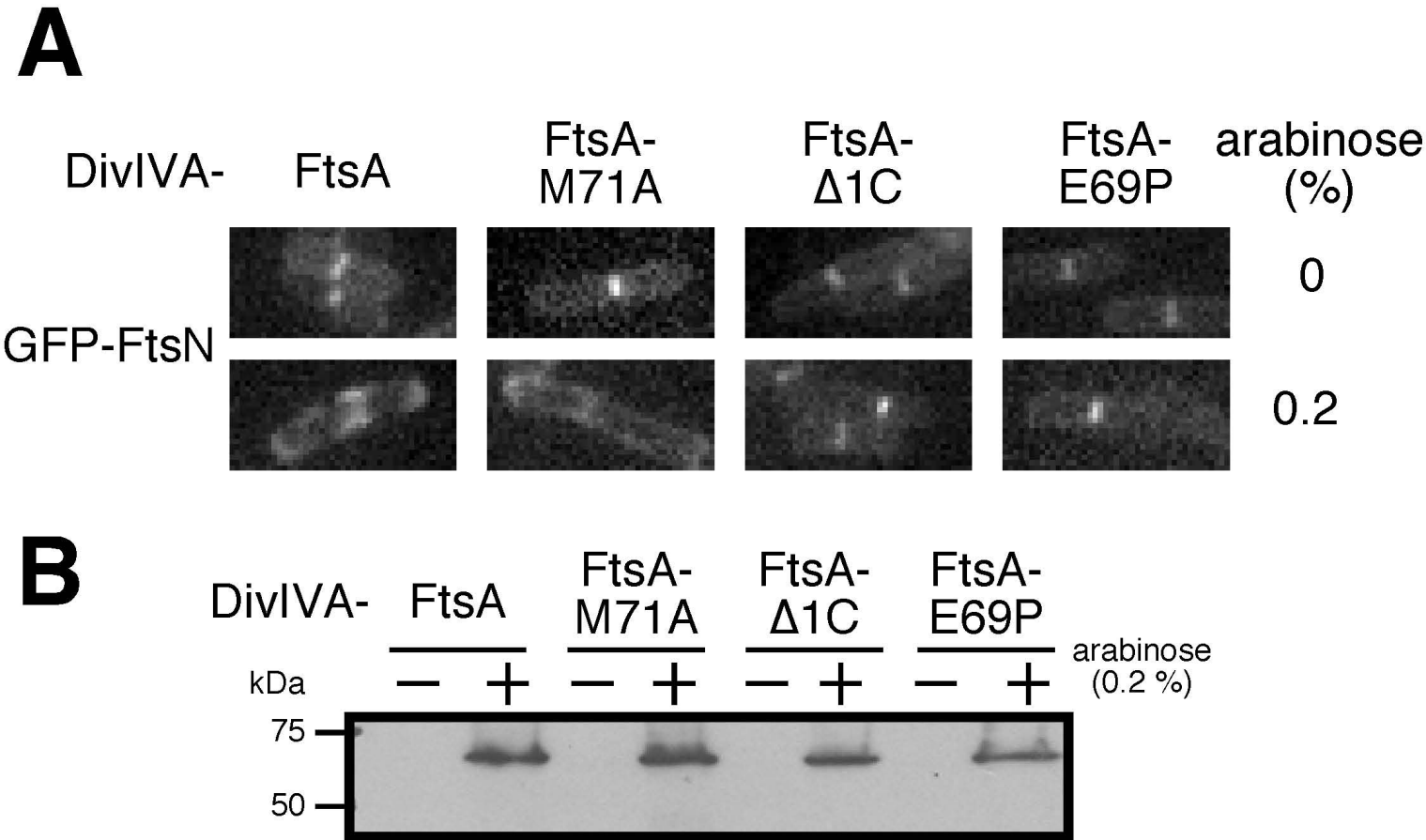
Fig. S2 Relocalization of GFP-FtsN to cell poles by FtsA derivatives fused to DivIVA. (A) Localization of GFP-FtsN in WM1074 cells carrying pWM1806 (DivIVA-FtsA), pWM3070 (DivIVA-FtsA-M71A), pWM3071 (DivIVA-FtsA-Δ1C), or pWM3072 (DivIVA-FtsA-E69P) in the absence and presence of 0.2% arabinose. (B) Immunoblot of representative cells used for microscopic experiments, probed with anti-DivIVA antibody.

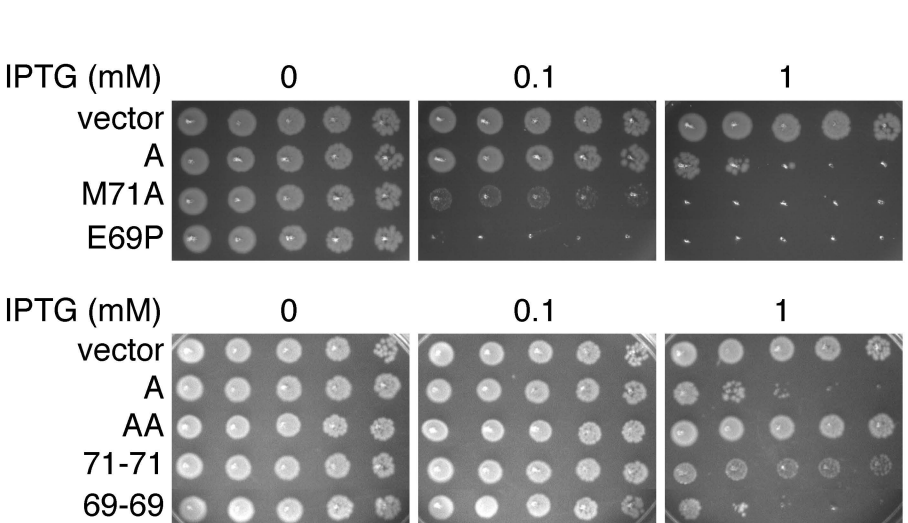
Fig. S3. Toxicity of overproduced monomeric or tandem dimer fusions of FtsA in WT cells. (A) Toxicity of overproduced FtsA derivatives is shown by colony viabilities of WM1074 carrying pWM2780 (vector), pWM2669 (FtsA), pWM2671 (FtsA-M71A), or pWM3055 (FtsA-E69P) in the presence of the indicated concentrations of IPTG. (B) Toxicity of overproduced tandem FtsA dimer derivatives is shown by colony viabilities of WM1074 carrying pWM2780 (vector), pWM2669 (FtsA), pWM3079 (AA), pWM3082 (71-71), or pWM3085 (69-69) in the presence of the indicated concentrations of IPTG.

Fig. S4. Sequence alignment of alpha-helix H1 of FtsA from different species. Conserved Glu69 and Met71 are shown in bold.

Fig. S5. Complementation of an FtsA depletion strain (WM1281) by FtsA derivatives. WM1281 containing plasmids producing FtsA (pWM2669), FtsA-M71A (pWM2671), FtsA* (pWM2781), or FtsAA (pWM3079) were grown at 30˚C for 4 h in the absence of IPTG prior to spotting on plates in the presence of the indicated concentrations of IPTG, which were then incubated overnight at 42˚C.


30˚C
42˚C
GFP-FtsA
GFP-FtsA-M71A
GFP-FtsA-E69P


A
DivIVA-
FtsA
FtsA-M71A
FtsA-Δ1C
FtsA-E69P
arabinose (%)
GFP-FtsN
0
0.2
B
DivIVA-
FtsA
FtsA-M71A
FtsA-Δ1C
FtsA-E69P
arabinose (0.2 %)
kDa
– + – + – + – +
75
50


A
IPTG (mM)
0
0.1
1
vector
A
M71A
E69P
B
IPTG (mM)
0
0.1
1
vector
A
AA
71-71
69-69

69 71

E.coli	LESVVKCVQRAIDQA**E**L**M**A	72
D.radiodurans	LERATQAIKQSLHAA**E**RVS	69
N.meningitidis	IDATVQAIRQAVNDA**E**L**M**A	65
B.mallei	IEATVQSIQRALEEA**E**L**M**A	71
M.capsulatus	LETTVHSIQRAVEEA**E**L**M**A	72
X.campestris	IESTVQSIQRAVEEA**E**L**M**A	72
B.bacteriovorus	IEATTDSIRKAKEEA**E**L**M**S	63
C.psychrerythraea	LNLVIQAIQRAINEA**E**L**M**A	72
B.burgdorferi	IEAALDSISNSIEAA**E**LIS	68
P.multocida	LNAVVTSVQRAIELA**E**SVA	72
H.influenzae	LDAVVGSIQRAIEAA**E**S**M**A	72
I.loihiensis	LNLVVQSVQRAVDEA**E**L**M**A	64
L.lactis	IEKVAQALRKAVNAA**E**ERA	68
E.faecium	IDKTVQAIQRAVRQA**E**EKA	68
B.subtilis	IDETVHSIRKAFDQA**E**R**M**V	68
C.jejuni	IELASKSIEEAVRSA**E**M**M**S	62
F.johnsoniae	ITQTIQSIQQAILEA**E**NNS	69
R.typhi	LKNAETSILTAIYAL**E**KDC	63
C.crescentus	LDEAAQAIAQAVERA**E**TVA	66
Z.mobilis	MESTEKAVREAVEQA**E**RIA	71
P.gingivalis	IDEAAAIIRRIVNQLNENL	71
P.putida	IESTVQSIQRAVEEAQL**M**A	65
C.tetani	IDETSEGIKTSIYQLQT**M**I	64
H.hepaticus	IELASLAIRNSVNDAKRVA	68
R.sphaeroides	MNETERAIRTAVQAAQK**M**A	90
S.aureus	FDIARQAIKDTIKKASIAS	63
T.maritima	AIAFKESVNTLLKEL**E**EQL	71

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