A Novel RNA Polymerase-Binding Protein Controlling Genes Involved in Spore Germination in *Bacillus subtilis*

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Supporting Information

(SI Experimental Procedures; SI References; Tables S1-2; Figures S1-3)

15 **SI Experimental Procedures**

16 **Plasmid construction**

17 pAH125 (*amyE*::P_{sspB}-*lacZ*) was constructed as previously described (Camp & Losick, 2009).
18 pBAT84 (*amyE*::P_{spoVT}-*lacZ*) was generated by cloning a *Eco*RI and *Bam*HI digested PCR
19 fragment containing the *spoVT* promoter into pAH124 (Camp & Losick, 2009) digested with the
20 same restriction enzymes. pBAT151 (*sacA*::*ylyA*) was constructed as previously described
21 (Traag *et al.*, 2013). pBAT260 (*sacA*::P_{sspB}-*ylyA*) was generated by fusing a PCR fragment
22 containing the *sspB* promoter and ribosome binding site (RBS) with a PCR fragment containing
23 the open reading frame (ORF) of *ylyA* by overlapping PCR. The resulting fragment was then
24 digested with *Eco*RI and *Bam*HI, and cloned into pSac-Cam (Middleton & Hofmeister, 2004)
25 digested with the same restriction enzymes. pBAT298-300 (P_{sspB}-directed overexpression of
26 *yocK, yteA* and *dksA*) were generated in the same way. pRLG10611 was generated by cloning a
27 *Xba*I and *Xho*I digested PCR fragment containing the *ylyA* ORF into pET28a (Novagen),
28 resulting in a construct for the expression of C-terminal, hexahistidine-tagged YlyA.

29

30 **Protein expression and purification**

31 *E. coli* BL21 (DE3) derivative strains carrying plasmids pRLG10611 (see above), pDP90
32 (Kearns *et al.*, 2005), pRL3034 (Losick lab strain collection) or pMF327 (Fujita & Sadaie,
33 1998), encoding hexahistidine-tagged YlyA, SinI, σ^G and σ^A, respectively, were grown at 25˚C
34 in 1L Luria Bertani (LB) broth. When the cultures reached an OD_{600} of 0.6-0.8, protein
35 expression was induced by the addition of 100 µM isopropyl β-D-1-thiogalactopyranoside
36 (IPTG). Cells were harvested and pelleted after four hours of additional growth at 25˚C, and cell
37 pellets were frozen overnight at -80˚C. The next day cell pellets were resuspended in lysis buffer

38 (50 mM Tris-Cl pH 8.0, 300 mM NaCl, 5 mM imidazole, 1 mM DTT, 1 mM
39 phenylmethanesulfonylfluoride (PMSF)), and cells were lysed by sonication. Cell debris was
40 removed by centrifugation, and the supernatants were incubated with Ni-NTA agarose beads
41 (Qiagen) at 4˚C for 30 minutes. The Ni-NTA agarose-cell suspension was then applied to a poly-
42 prep chromatography column (Biorad). The resulting Ni-NTA column was consecutively washed
43 with 10 column volumes of lysis buffer, one column volume of buffer containing 25 mM
44 imidazole, and one column volume of buffer containing 50 mM imidazole. Proteins were eluted
45 from the column with column volume fractions of buffer containing 200 mM imidazole. The
46 elution fractions were run on a SDS-PAGE gel, and appropriate fractions were dialyzed at 4˚C
47 overnight against 1L storage buffer (25 mM Tris-Cl pH 8.0, 100 mM NaCl, 50% glycerol, 1 mM
48 DTT).
49 For purification of *B. subtilis* RNA polymerase core, approximately 500 bp of the 5' end
50 of *rpoC*, excluding the stop codon, was cloned *Nde*I and *Xho*I into pET28a, resulting in a
51 truncated *rpoC* gene in frame with a sequence coding for six histidine residues. This plasmid-
52 borne fragment and an approximately 500 bp genomic DNA fragment downstream of *rpoC* were
53 amplified by PCR, using primers containing sequences complementary to a spectinomycin
54 resistance cassette. The resulting fragments and outer primers were then used to amplify a
55 spectinomycin resistance cassette. *ylyA* mutant cells were transformed with this double stranded
56 linear DNA fragment, and double cross-over events were selected by plating on LB agar
57 containing spectinomycin. The resulting strain (RL5493) encodes a C-terminal, hexahistidine-
58 tagged β' subunit as the only copy in the cell. This strain was grown in 1L LB broth at 37˚C until
59 an OD_{600} of 1, at which point cells were harvested and pelleted. The pellet was resuspended
60 immediately in buffer containing 300 mM NaCl, 50 mM Na_2HPO_4, 3 mM 2-mercaptoethanol, 5

61 % glycerol, and 1 mM PMSF. RNA polymerase core was purified using Ni-NTA agarose similar
62 to previously described (Anthony *et al.*, 2000). Fractions eluted from the Ni-NTA column were
63 run on a SDS-PAGE gel, and appropriate fractions were dialyzed at 4˚C overnight against buffer
64 containing 50 mM Tris-Cl pH 8.0, 0.1 M NaCl, 3 mM 2-mercaptoethanol, 50% glycerol. RNA
65 polymerase preparations were stored at -20˚C.

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67 **SI References**

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81 which foreign DNA may be inserted without affecting transposition in Bacillus subtilis or
82 expression of the transposon-borne erm gene. *Plasmid* **12**: 1-9.

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86 **Table S1.** ***B. subtilis*** **strains used in this study.**

Strain[a]	Genotype	Source or reference
PY79	Prototrophic derivative of *B. subtilis subsp. subtilis* 168	(Youngman *et al.*, 1984)
RL5371	*ylyA::erm*	(Traag et al., 2013)
RL5372	*ylyA::erm sacA::ylyA* (*kan*)	(Traag et al., 2013)
RL5480	*ylyA::erm sacA::*P_{sspB}-*ylyA* (*cam*)	This study
RL5481	*amyE::*P_{sspB}-*lacZ* (*neo*)	This study
RL5482	*ylyA::erm amyE::*P_{sspB}-*lacZ* (*neo*)	This study
RL5483	*ylyA::erm sacA::ylyA* (*kan*) *amyE::*P_{sspB}-*lacZ* (*cat*)	This study
RL5484	*ylyA::erm sacA::*P_{sspB}-*ylyA* (*cat*) *amyE::*P_{sspB}-*lacZ* (*neo*)	This study
RL5485	*amyE::*P_{spoVT}-*lacZ* (*neo*)	This study
RL5486	*ylyA::erm amyE::*P_{spoVT}-*lacZ* (*neo*)	This study
RL5487	*ylyA::erm sacA::*P_{sspB}-*ylyA* (*cat*) *amyE::*P_{spoVT}-*lacZ* (*neo*)	This study
AHB1449	*ywrK*::Tn*917*::*amyE*::P^{T7}-*lacZ* (*neo*) *ylnF*::Tn*917*::*amyE*::P_{spoIIQ}-*T7RNAP* (*spec*)	(Camp & Losick, 2009)
RL5488	*ylyA::erm ywrK*::Tn*917*::*amyE*::P^{T7}-*lacZ* (*neo*) *ylnF*::Tn*917*::*amyE*::P_{spoIIQ}-*T7RNAP* (*spec*)	This study
RL5489	*ylyA::erm sacA::*P_{sspB}-*ylyA* (*cat*) *ywrK*::Tn*917*::*amyE*::P^{T7}-*lacZ* (*neo*)	This study

	ylnF::Tn*917*::*amyE*::P_{spoIIQ}-*T7RNAP* (*spec*)	
RL5490	*ylyA::erm sacA::*P_{sspB}-*yocK* (*cat*) *amyE::*P_{sspB}-*lacZ* (*neo*)	This study
RL5491	*ylyA::erm sacA::*P_{sspB}-*yteA* (*cat*) *amyE::*P_{sspB}-*lacZ* (*neo*)	This study
RL5492	*ylyA::erm sacA::*P_{sspB}-*dksA* (*cat*) *amyE::*P_{sspB}-*lacZ* (*neo*)	This study
RL5493	*ylyA::erm rpoC::rpoC-6His* (*spec*)	This study

87 [a]All strains were derived from the prototrophic laboratory strain PY79.

88

89 **Table S2. Relative levels of germination proteins in spores from wild type, *ylyA* mutant,**

90 **and *ylyA* overexpression strains.**

	wild type	Δ*ylyA*	P_{sspB}-*ylyA*
GerAA	1	1	0.22
GerAC	1	0.85	0.13
GerBC	1	0.25	0.12
GerKA	1	1	0.25
SpoVAD	1	2.5	1
GerD	1	1	1

91 *All values are averages of results from replicate western blot analysis of two independent spore

92 preparations, and these values differed by ≤ 20%. Values for wild type spores have been set at 1,

93 and values for other strains are expressed relative to these values.

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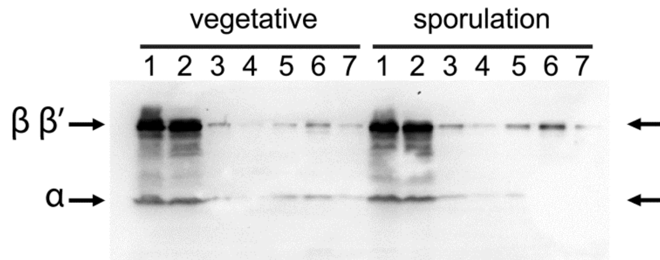

Fig. S1. YlyA interacts with RNA polymerase from lysate from vegetative and sporulating cells. Western blot analysis of the co-immobilization of RNA polymerase subunits with hexahistidine-tagged YlyA. RNA polymerase was similarly co-immobilized when lysates from vegetative (left) and sporulating (right) *ylyA* mutant cells were used. Lanes: soluble protein lysate (1), flow through (2), first wash (3), final wash (4) elution fractions (5-7). Arrows indicate bands corresponding to the β/β' and α subunits of RNA polymerase which co-eluted with YlyA.

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YlyA_Bamy       MNDQLTAIHTELLMMKEELQSRLFEYSCFHMSASV-----EPSMND-QEATLIYHIKEEL
YlyA_Bsub       MNDQLTAIYTELLLMKEELQSRLFEYSCFQVSTSP-----QAAINQKQKATLIYHIKEEL
YlyA_Blic       MNEQLAAIHTELLQMKEELEARLFEYSFFQQDS--------QGISSYQQATLMYHVKEEL
YlyA_Bpum       MIGDLETIYTELLQMKEELQAKLFEYTSFRSPAEH------VSMNEVQTATLLYHIKEEL
YlyA_Bcer       MNEMYMEIKEELQLMRKELQERLAKEVMYKYNTEFSEELGYEMKEEIKKKLLLHDIKEDL
YlyA_Bwei       MNEIYMEIKEELQLMRKELQDRLAKEVMHTYYMEFGQELGYEIKEETKKKLLLHDIKEDL
                *      *  **  *::**: :* :   .               .. :   *::.:**:*

YlyA_Bamy       QDVLLALSKFENNMYGYCEKTGDPIPIEKLAVLPTARTADDFYYPAQFEKKTLP-YWEPG
YlyA_Bsub       QDVLLALSKIENGTFGYCEETGAPIPLAKLAVLPTARTANDFLYSVQFEKKTLP-IWKST
YlyA_Blic       QDVLLALSKIENGTYGICEETNQAIPLAKMTILPTARTANDFLYHAQFEKKTLP-IWAET
YlyA_Bpum       QDVSLAIAKIEQGTYGICEATGDVIPLEQMSILPTARTVDDFLYHKQYEKKAFTPYPHES
YlyA_Bcer       KDVERALFKMEIDMYGICEDTGRVISVKQMKTMPTARTIHEFFYEKVNV-----------
YlyA_Bwei       KDVERALFKMEIDMYGICEETGRAISTKQMKTMPTARTIHEFFYEKVNV-----------
                :**  *: *:* . :* ** *.  *.  ::  :***** .:* *

YlyA_Bamy       DYAYDQALYE
YlyA_Bsub       DIEYGQALYE
YlyA_Blic       DETFNRALYS
YlyA_Bpum       DDSHFEAFHM
YlyA_Bcer       ----------
YlyA_Bwei       ----------
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Fig. S2. Alignment of the amino acid sequences of YlyA orthologs from *Bacillus* species.

ClustalW was used to align the amino acid sequences of YlyA orthologs from *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. cereus*, and *B. weihenstephanensis*. Identical residues (*), conserved substitutions (:), and semi-conserved substitutions (.) are indicated below the alignment. The region around the predicted coiled-coil tip is indicated with a hook above the sequence.

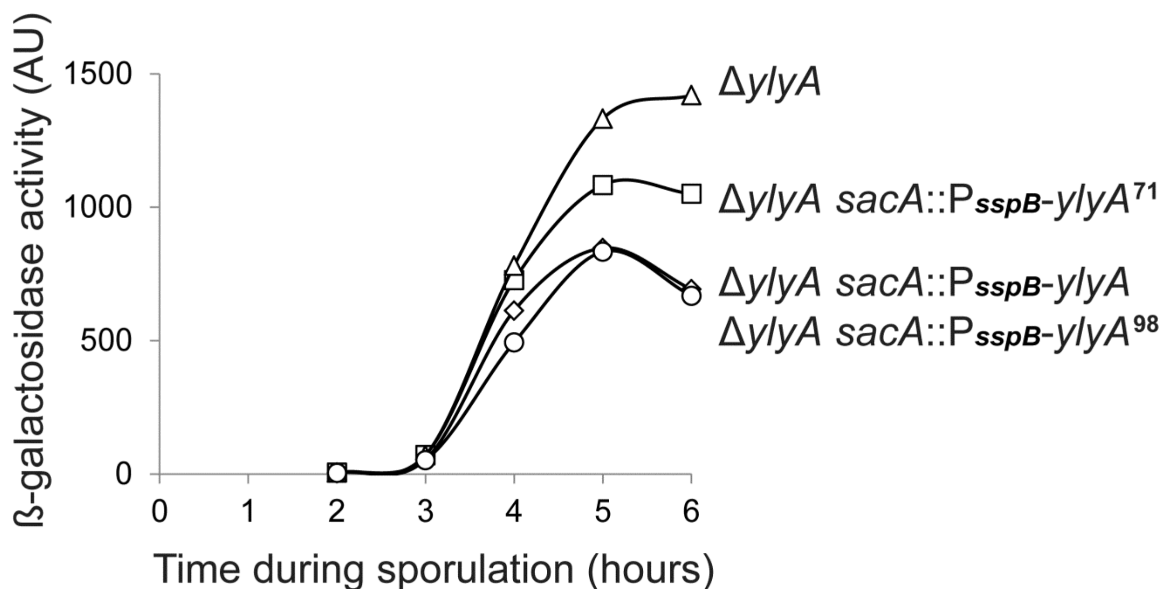

Fig. S3. The C-terminus of YlyA is partially dispensible *in vivo*. β-galactosidase activity (AU) was monitored for samples taken at the indicated time points after sporulation was induced by resuspension. P_{sspB}-directed *lacZ* activity was determined in *ylyA* mutant cells (triangles), *ylyA* mutant cells carrying a copy of *ylyA* expressed from the *sspB* promoter at the ectopic *sacA* locus (circles), and *ylyA* mutant cells carrying C-terminally truncated copies of the *ylyA* gene encoding YlyA residues 1-98 (*ylyA*^{98}; diamonds) and YlyA residues 1-71 (*ylyA*^{71}; squares), expressed from the *sspB* promoter at the ectopic *sacA* locus. All strains carry the P_{sspB}-*lacZ* reporter construct at the *amyE* locus.