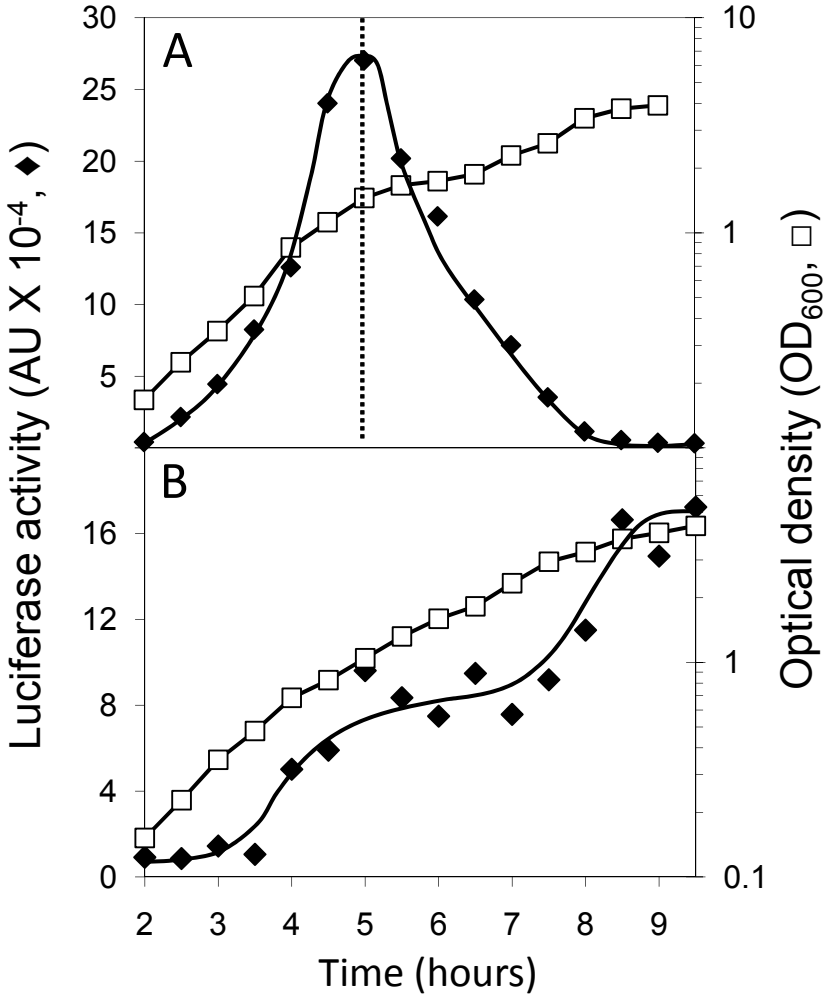
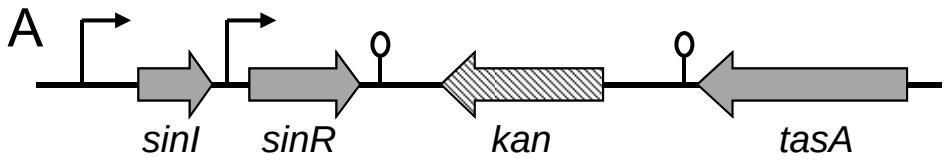
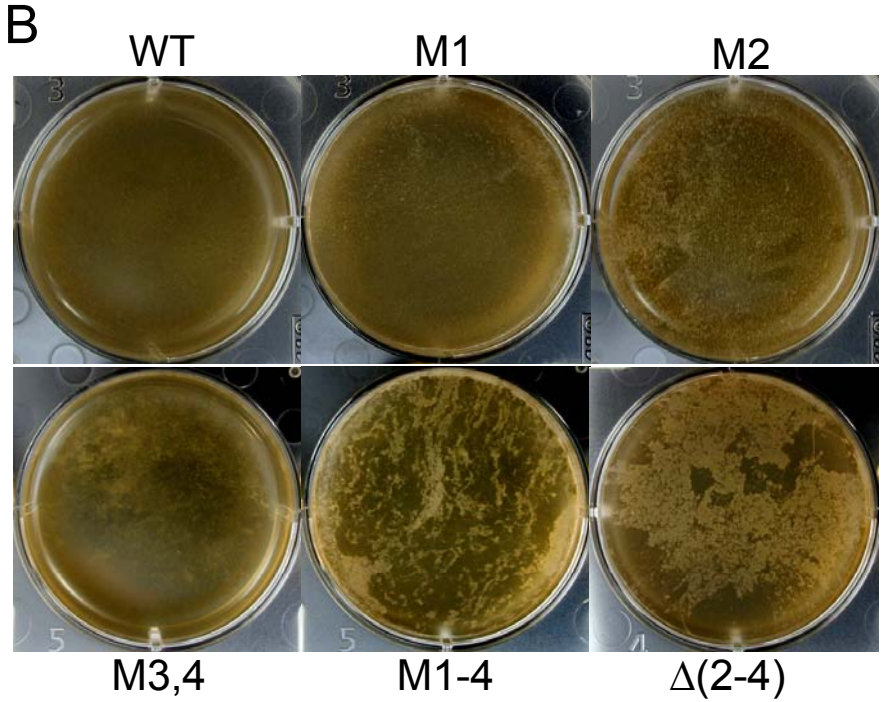


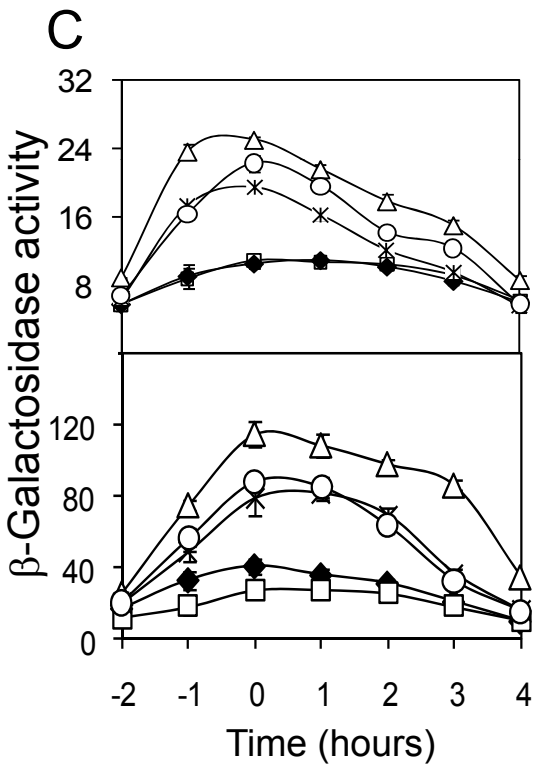
Supplemental Figure 1

Supplemental Figure Legends

Figure S1. *sinI* expression is sustained in biofilm but not sporulation medium. Shown are luciferase activities from a 3610 derivative (ALM66) harboring a P_{sinI}-*lux* fusion and cultured in either DS (A) or MSgg (B) medium. Growth curves of the cells were shown in open squares (□) and luciferase activities were shown in filled diamonds (♦) and in arbitrary units (AU).

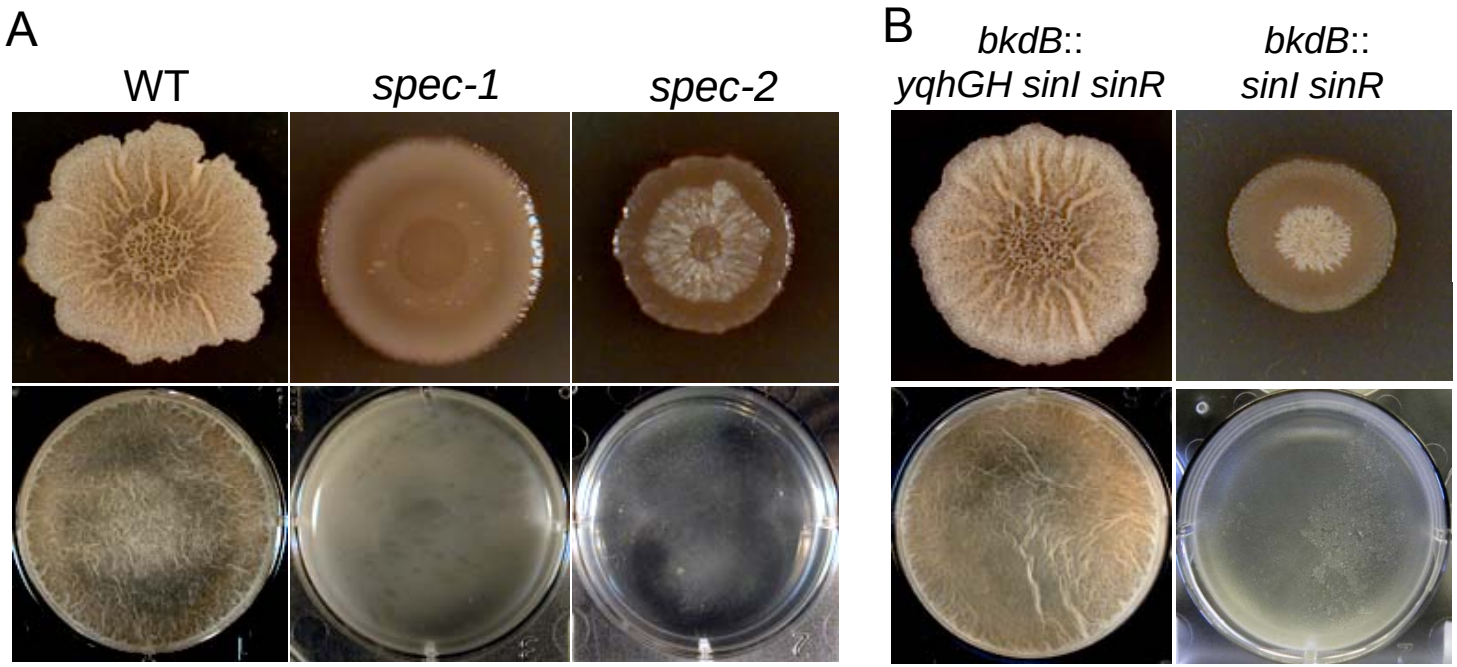






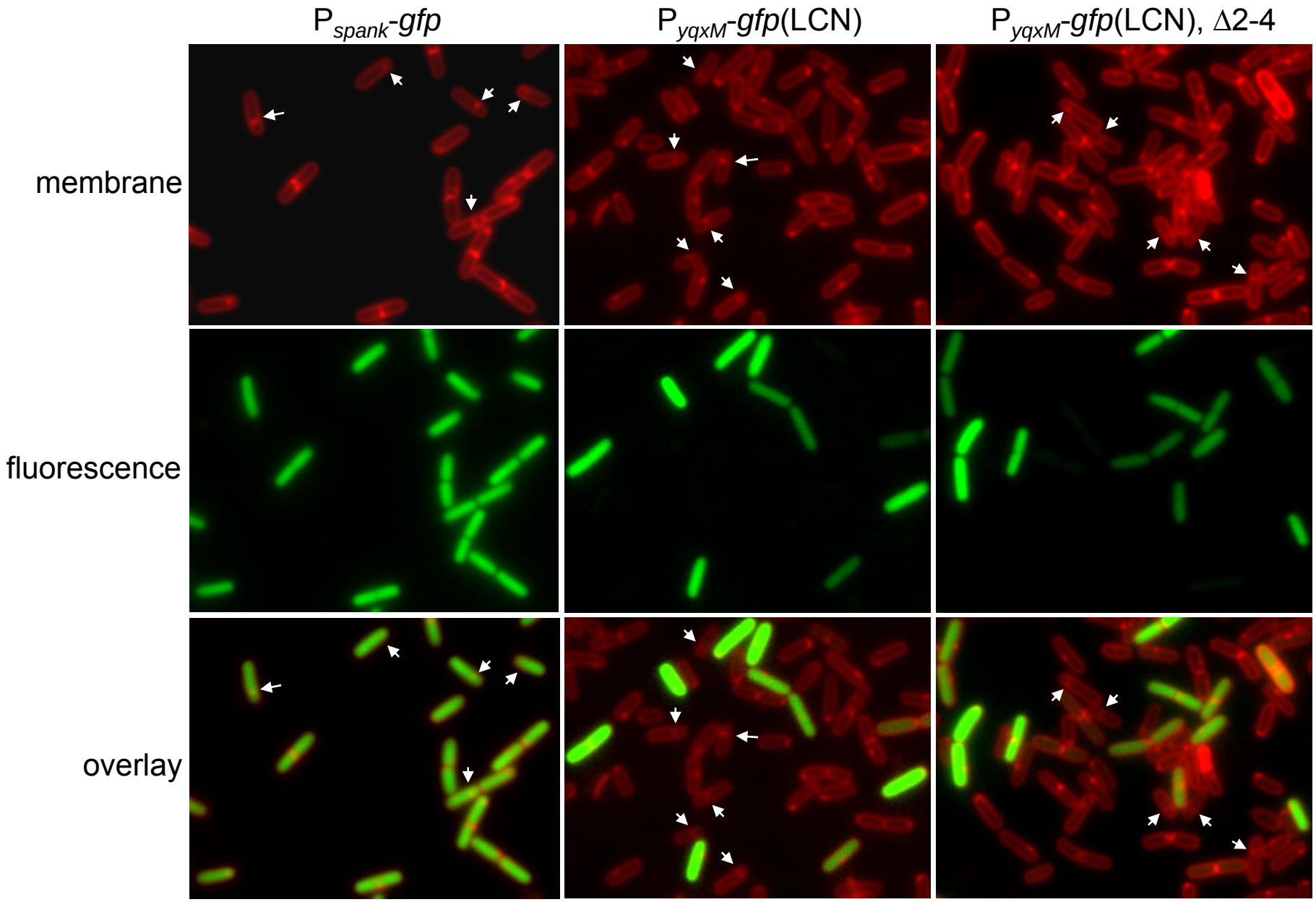
Supplemental Figure 2

Figure S2. (A) A schematic drawing shows insertion of *kan* selective marker to a chromosomal locus between *sinR* and *tasA*. (B) Pellicle formation in DS medium by 3610 derivatives that contain mutant alleles in the putative 0A~P operators in the native *sinI* promoter. (C) β-Galactosidase activities of 3610 derivatives that contain either P_{epsA}-*lacZ* (upper panel) or P_{yqxM}-*lacZ* (lower panel) at the *amyE* locus on the chromosome. Cells were grown in DS medium. Each reporter strain also contains either wild type (♦), M1 allele (O), M2 allele (∗), M3, 4 allele (□), or Δ(2-4) (Δ) in the native *sinI* promoter.



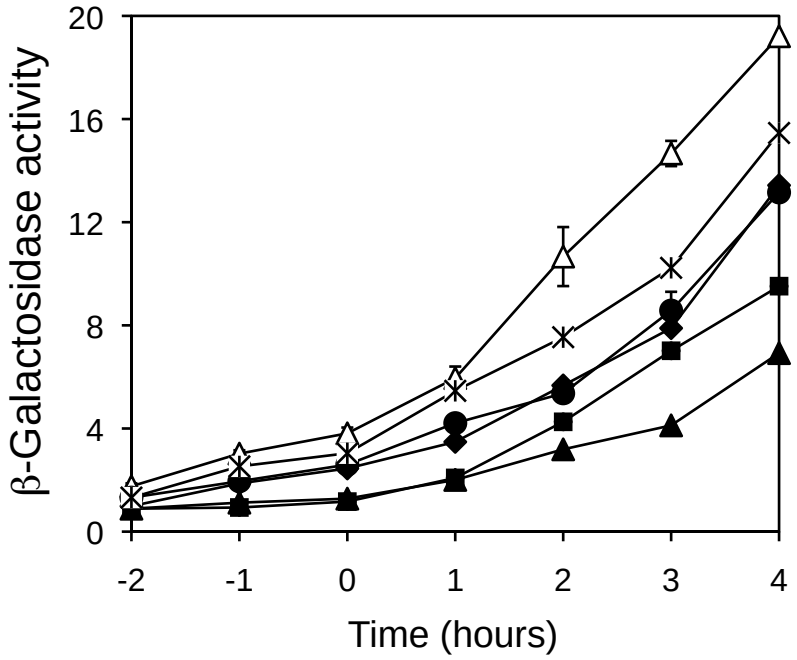
Supplemental Figure 3

Figure S3. Block of transcription read-through into *sinI* from upstream *yqhHG* operon impairs biofilm formation. (A) Colony and pellicle formation by 3610 (left-hand panel) and its derivatives (middle and right-hand panels) in which the *spec* gene was inserted upstream of *sinI* in two different loci. (B) Colony and pellicle formation by a Δ*sinI sinR* mutant complemented with a DNA fragment containing either *yqhHG sinI sinR* (left-hand panels) or *sinI sinR* (right-hand panels) and integrated at the *bkdB* gene locus.



Supplemental Figure 4

Figure S4. Matrix genes were shut off in early sporulating cells. In the left-hand panels, green fluorescence represents YC161 cells expressing GFP from a constitutive promoter (P_{spank}-*gfp*). Green fluorescence in the middle and right-hand panels represents YC716 (middle panels) and YC717 (right-hand panels, ∆2-4) cells, respectively, expressing proteolytically unstable GFP proteins (GFP-LCN) from the promoter for the *yqxM* operon. The asymmetric division septums in early sporulating cells were indicated by arrows. In all panels, red fluorescence represents cell membrane staining using the dye FM4-64.



Supplemental Figure 5

Figure S5. β-Galactosidase activities of 3610 containing either a wild type P_{sinI}-*lacZ* fusion or P_{sinI}-*lacZ* with mutations in the imperfect 0A-box. Strains used were described as follows: wild type (■), or M1 allele (●), or M2 allele (♦), or M3-4 allele (▲) or Δ(2-4) allele (Δ), or random sequence shuffle of O(2-4) (∗) in the promoter region of P_{sinI}-*lacZ*. Reporter fusions were integrated at *amyE* on the chromosome of 3610 and cells were grown in MSgg medium.

Table S1. Strains used in the study.

E. coli		
DH5α	an *E. coli* strain used for molecular cloning	Invitrogen
BL21/DE3	*E. coli* B F^- *dcm ompT hsdS*(rB^- mB^-) *gal* λ(DE3)	Stratagene
RL3514	a BL21/DE3 derivative for overexpression of Spo0A-CTD Cm^R, Amp^R	(Molle et al, 2003)
B. subtilis		
3610	wild *B. subtilis* strain capable of forming robust biofilms	(Branda et al, 2001)
PY79	laboratory strain used as a host for transformation	
AHB277:	*Tn917*@140°(*ylnF/yloA*)::*amyE*::P_{spoIIG}-*sigG*::*spec*, $Spec^R$	gift of Camp A
AHB289:	*Tn917*@317°(*ywrK*)::*amyE*::P_{spoIIG}-*sigG*::*spec*, $Spec^R$	gift of Camp A
AHB290:	*Tn917*@213°(*bkdB*)::*amyE*::P_{spoIIG}-*sigG*::*spec*, $Spec^R$	gift of Camp A
ALM66	P_{sinI}-*lux* at *sacA*, Δ*epsH*::*tet*, $Spec^R$, Tet^R	gift of McLoon A
DL841	*lacA*::P_{yqxM}-*yfp*, Mls^R	gift of Lopez D
RL3618:	*spo0A*Ω*spo0A-sad67*::*spec*, $Spec^R$	(Ireton et al, 1993)
RL3852:	Δ*epsH*::*Tet,* Tet^R	(Kearns et al, 2005)
RL3855:	Δ*sinI sinR*::*spec,* $Spec^R$	(Kearns et al, 2005)
YC161:	*amyE*::P_{spank}-*gfp*, Cm^R	this study
YC256:	P_{sinI}-*lacZ* at *amyE*, Cm^R	this study
YC256(M1):	*amyE*::P_{sinI}-*lacZ*, operator mutation in O1, Cm^R	this study
YC256(M2):	*amyE*::P_{sinI}-*lacZ*, operator mutation in O2, Cm^R	this study
YC256(M3, 4):	*amyE*::P_{sinI}-*lacZ*, operator mutations in O3 and O4, Cm^R	this study
YC256(Δ2-4):	*amyE*::P_{sinI}-*lacZ*, deletions from O2 to O4, Cm^R	this study
YC256(RS2-4):	*amyE*::P_{sinI}-*lacZ*, scrambled sequence from O2 to O4, Cm^R	this study
YC264:	*spec* gene inserted downstream of *yqhG*, $Spec^R$	this study
YC270:	Δ*sinI sinR*::*spec*, *yqhHG-sinI-sinR* at *amyE*, Cm^R, $Spec^R$	this study
YC285:	*kan* gene inserted between *sinR* and *tasA*, Kan^R	this study
YC285(M1):	mutations in 0A~P operator 1 in YC285, Kan^R	this study
YC285(M2):	mutations in 0A~P operator 2 in YC285, Kan^R	this study
YC285(M3, 4):	mutations in 0A~P operators 3 and 4 in YC285, Kan^R	this study
YC285(M1-4):	mutations in 0A~P operators 1, 2, 3 and 4 in YC285, Kan^R	this study
YC285(Δ2-4):	deletions from 0A~P operators 2 to 4 in YC285, Kan^R	this study
YC290:	*amyE*::P_{epsA}-*lacZ* in YC285, Cm^R, Kan^R	this study
YC290(M1):	mutations in 0A~P operator 1 in YC290, Cm^R, Kan^R	this study
YC290(M2):	mutations in 0A~P operator 2 in YC290, Cm^R, Kan^R	this study
YC290(M3, 4):	mutations in 0A~P operators 3 and 4 in YC290, Cm^R, Kan^R	this study
YC290(Δ):	deletions in 0A~P operators 2-4 in YC290, Cm^R, Kan^R	this study
YC291:	*amyE*::P_{yqxM}-*lacZ* in YC285, Cm^R, Kan^R	this study
YC291(M1):	mutations in 0A~P operator 1 in YC291, Cm^R, Kan^R	this study
YC291(M2):	mutations in 0A~P operator 2 in YC291, Cm^R, Kan^R	this study
YC291(M3, 4):	mutations in 0A~P operators 3 and 4 in YC291, Cm^R, Kan^R	this study
YC291(Δ):	deletions in 0A~P operators 2-4 in YC291, Cm^R, Kan^R	this study
YC550:	Δ*sinI sinR*::*spec*, *amyE*::*yqhHG-sinI-sinR* at 140°(*ylnF/yloA*), Cm^R, $Spec^R$	this study
YC551:	Δ*sinI sinR*::*spec*, *amyE*::*yqhHG-sinI-sinR* at 317°(*ywrK*), Cm^R, $Spec^R$	this study
YC552:	Δ*sinI sinR*::*spec*, *amyE*::*yqhHG-sinI-sinR* at 213°(*bkdB*), Cm^R, $Spec^R$	this study
YC553:	*spec* gene inserted immediately upstream of *yqhH*, $Spec^R$	this study
YC561:	*amyE*::P_{sinI}-*lacZ* ($0A^{mut}$), Cm^R	this study
YC562:	*amyE*::*yqhHG-sinI-sinR* at 213°(*bkdB*), Cm^R	this study
YC686:	*thrC*::P_{yqxM}-*lacZ* in 3610, Mls^R	this study
YC687:	Δ*sinI sinR*::*spec*, *amyE*::*yqhHG-sinI-sinR*, *thrC*::P_{yqxM}-*lacZ*, $Spec^R$, Cm^R, Mls^R	this study
YC688:	*amyE*::*yqhHG-sinI-sinR* at 213°(*bkdB*), *thrC*::P_{yqxM}-*lacZ*, Cm^R, Mls^R	this study
YC716:	Δ*epsH*::*tet*, *amyE*::P_{yqxM}-*gfp*(LCN), *kan* gene inserted between *sinR* and *tasA* Tet^R, Cm^R, Kan^R	this study
YC717:	Δ*epsH*::*tet*, *amyE*::P_{yqxM}-*gfp*(LCN), *kan* gene inserted between *sinR* and *tasA* deletions in 0A~P operators 2-4, Tet^R, Cm^R, Kan^R	this study
YC718:	*spo0A*Ω*spo0A-sad67*, *kan* gene inserted between *sinR* and *tasA*, deletions in 0A~P operators 2-4, *lacA*::P_{yqxM}-*yfp*, $Spec^R$, Kan^R, Mls^R	this study
YC719:	*spo0A*Ω*spo0A-sad67*, Δ*sinI-sinR*::*kan*, *amyE*::*yqhHG-sinI-sinR* deletions in 0A~P operators 2-4, *lacA*::P_{yqxM}-*yfp*, $Spec^R$, Kan^R, Cm^R Mls^R	this study
YC734:	*amyE*::P_{spoIIA}-*gfp*, *sacA*::P_{yqxM}-*lux*, $Spec^R$, Cm^R	this study
YC750:	Δ*sinI sinR*::*spec*, *amyE*::*sinI-sinR* at 213°(*bkdB*), Cm^R, $Spec^R$	this study

Table S2. Plasmids and primers used in the study.

Plasmids:		
pAH49	pBSK(+) derivative, Kan^R, Amp^R	gift of Camp A
pAH54	pBSK(+) derivative, $Spec^R$, Amp^R	gift of Camp A
pDG268	*amyE* integration vector, promoter-less *lacZ*, Cm^R	(Antoniewski et al, 1990)
pDG1662	*amyE* integration vector, Cm^R	(Guérout-Fleury et al, 1996)
pDG1730	*amyE* integration vector, $Spec^R$	(Guérout-Fleury et al, 1995)
pNGFP	vector with a promoter-less *gfp* for transcriptional or translational fusion	(Arnaud et al, 2004)
pYC166	*amyE*::P_{sinI}-*lacZ*, Cm^R	this study
pYC166(Δ2-4)	pYC166 derivative, deletion of 0A~P operator sites 2-4	this study
pYC171	1-kb DNA upstream and downstream of the stop of *yqhG* cloned into pAH54 to flank the *spec* gene, $Spec^R$	this study
pYC191	a pDG1662 derivative, *amyE*::*yqhHG sinI sinR*, Cm^R	this study
pYC202	1-kb DNA upstream and downstream of the start of *yqhH* cloned into pAH54 to flank the *spec* gene, $Spec^R$	this study
pYC213	a pDG1662 derivative, *amyE*::P_{yqxM}-*gfp-LCN*, Cm^R	this study
pYC237	a pDG1730 derivative, *amyE*::P_{spoIIA}-*gfp*, $Spec^R$	this study

Primers:

gfp-F1:	5′-gacaagcttggtaccgggcccccctcgaggt-3′
gfp-R1:	5′-gacggatccgagctcctatttgtatagttcatccatgcc-3′
gfp-R2(LCN):	5′-gacggatccctagttgcacagtttgtatagttcatccatgcc-3′
P_{epsA}-F1:	5′-gtcgaattcctagaaattctcctctattcctgtcg-3′
P_{epsA}-R1:	5′-gtcaagcttctagacaatcgcatataattctttg-3′
P_{sinI}-F1:	5′-gacgaattcactgacgtctcaaatatgtg-3′
P_{sinI}-R1:	5′-gtcggatcccttcatgcagtttctcctccta-3′
P_{sinI}-MF1:	5′-attctcgtttttggtgtgaaaatacgattat-3′
P_{sinI}-MR1:	5′-ataatcgtattttcacaccaaaaacgagaat-3′
P_{sinI}-AF1:	5′-aacttttttaccattaaccctcattctcgtttttt-3′
P_{sinI}-AR1:	5′-aaaaaacgagaatgagggttaatggtaaaaaagtt-3′
P_{sinI}-ΔR1:	5′-gacaagctttatacctttattataatcgt-3′
P_{sinI}-ΔF1:	5′-gacaagcttgactaatgaagcatacaata-3′
P_{sinI}-MF2:	5′-aataaaggtataatgcaataaaattctgg-3′
P_{sinI}-MR2:	5′-ccagaattttattgcattatacctttatt-3′
P_{sinI}-MF3:	5′-ggtgatttaaacggaaatgacttccag-3′
P_{sinI}-MR3:	5′-ctggaagtcatttccgtttaaatcacc-3′
P_{sinI}-MF4:	5′-ggcaaatgacatcctgtgactaatgaag-3′
P_{sinI}-MR4:	5′-cttcattagtcacaggatgtcatttgcc-3′
P_{sinI}-RS-F:	5′-gctaagctttaacagtttctgtgtctaatgacataagtggca-3′
P_{sinI}-RS-R:	5′-agcaagcttttcttcattatgccacttatgtcattagacaca-3′
P_{sinI}-FP-F1:	5′-gctagaattctttatctgcaaaataatatttc-3′
P_{sinI}-FP-R1:	5′-gctaggatcccctaaaatacttgtttatgtat-3′
P_{sinI}-KO-P1:	5′-gactctcgaggaattcgtggtcggtgaacttgatga-3′
P_{sinI}-KO-P2:	5′-gatctcgagttagcttgaaatattatttttg-3′
P_{sinI}-KO-P3:	5′-gactggatccctttttttaccattcgacatca-3′
P_{sinI}-KO-P4:	5′-gactgagctctgaaaaagcgcatagcgagg-3′
P_{spoIIA}-F1:	5′-gcataagcttagagcttggcactcttgttctg-3′
P_{spoIIA}-R1:	5′-gtacaagcttgatatgatcggataatgag-3′
P_{yqhHG}-KO-P1:	5′-gactctcgaggaattccgtgaaataagtgctttgcg-3′
P_{yqHGH}-KO-P2:	5′-gactctcgagtgctttgaaaccgcctttct-3′
P_{yqhHG}-KO-P3:	5′-gactggatccatgaatacagaaatgatcta-3′
P_{yqhHG}-KO-P4:	5′-gactgagctctgtcacttgattgatgcgatct-3′
P_{yqhHG}-F1:	5′-gtacgaattcaaaggacagagaaacacctcatg-3′
P_{yqxM}-F1:	5′-gtacgaattcagacaaatcacacattgtttg-3′
P_{yqxM}-R1:	5′-gtacaagcttacctcctgtaaaacactg-3′
sinIR-R1:	5′-gacggatcctgaaaaagcgcatagcgagg-3′
tasA-F1:	5′-gatcctcgagtcaaaggatgctacttttgca-3′
tasA-R1:	5′-ccagcttttgttccctttagtgagttaatttttatcctcgctatgcg-3′
yqhH-F1:	5′-gtacgagctcgtgaacttgatgatattttaacg-3′
yqhH-R1:	5′-caattcgccctatagtgagtcgtataaaaattaataacagcaa-3′

Supplemental Materials and Methods

Strain and plasmid construction

1) Plasmid construction

pYC166 contains a P_{sinI}-*lacZ* transcriptional fusion in pDG268. pDG268 is an *amyE* locus integration vector which also contains a promoter-less *lacZ* gene (Antoniewski et al, 1990). To construct pYC166, the promoter region of *sinI* was amplified by PCR using 3610 chromosomal DNA as the template and primers P_{sinI}-F1 and P_{sinI}-R1. PCR products were gel-purified and cloned into the EcoRI and BamHI sites of pDG268, resulting in pYC166. Introduction of mutations to the regulatory region of *sinI* in pYC166 was achieved by site-directed mutagenesis as described above. To construct a 43 base-pair deletion spanning from the operators 2 to 4 in the regulatory region of *sinI*, two DNA fragments were amplified by PCR using primers P_{sinI}-F1 and P_{sinI}-MR1, and primers P_{sinI}-MF1 and P_{sinI}-R1, respectively. The first DNA fragment starts from the 5' end of the *sinI* promoter region and ends at the 5' end of operator site 2, and the second DNA fragment covers from 3' end of the operator site 4 to the immediate upstream of *sinI*. These two DNA fragments were cloned into pDG268 sequentially between the EcoRI and HindIII, and the HindIII and BamHI sites, and that resulted in a recombinant plasmid pYC166(∆2-4). To make a scrambled sequence for the operators 2 to 4, two overlapping oligonucleotides, P_{sinI}-RS-F1 and P_{sinI}-RS-R1, were used to generate a double strand DNA by overlapping extension in polymerization reaction. The DNA fragment was then cloned into the HindIII site of pYC166(∆2-4).

pYC191 contains a 3.5-kb *yqhHG sinI sinR* fragment cloned into pDG1662 (Guérout-Fleury et al, 1996). To construct pYC191, the above 3.5-kb DNA sequence was amplified by PCR using primers P_{yqhHG}-F1 and *sinIR*-R1. The PCR products were gel-purified and co-digested with EcoRI and BamHI. After co-digestion, the PCR products were turned into two smaller fragments. One starts from the promoter of *yqhH* and ends at an internal EcoRI site in *yqhH*, and the other starts from the internal EcoRI site to the end of *sinR*. Both DNA fragments were cloned into pDG1662 by three-fragment ligation, resulting in pYC191.

To construct pYC213, the promoter sequence of the *yqxM* matrix operon (P_{yqxM}) was amplified by PCR using primers P_{yqxM}-F1 and P_{yqxM}-R1 and 3610 chromosomal DNA as the template. The gene encoding a proteolytically unstable GFP protein (*gfp-LCN*) was also amplified by PCR using primers *gfp*-F1 and *gfp*-R2(LCN) and the plasmid pNGFP (Arnaud et al, 2004) as the template. Both PCR products were then digested and cloned into the plasmid pDG1662 sequentially, resulting in pYC213.

To construct pYC237, the promoter sequence of the *spoIIA* operon was amplified by PCR using using primers P_{spoIIA}-F1 and P_{spoIIA}-R1 and 3610 chromosomal DNA as the template. The *gfp* gene was amplified by PCR using primers *gfp*-F1 and *gfp*-R2, and the plasmid pNGFP as the template. Both PCR products were then cloned into pDG1730 (Guérout-Fleury et al, 1995) sequentially, resulting in pYC237.

2) Construction of YC256 and its derivatives

YC256 is a 3610 derivative that contains a transcriptional fusion of P_{sinI}-*lacZ* integrated into the *amyE* locus on the chromosome. To construct YC256, the recombinant plasmid pYC166 was first introduced into PY79 by transformation (Gryczan et al, 1978). Transformants were selected for integration of P_{sinI}-*lacZ* to the *amyE* locus in the chromosome of PY79 by double crossover recombination. The DNA fragment containing the P_{sinI}-*lacZ* transcriptional fusion was then introduced into 3610 by SSP1 phage-mediated transduction (Yasbin & Young, 1974). Introduction of P_{sinI}-*lacZ* with mutant alleles into 3610 chromosome was performed similarly as described above by using derivatives of the plasmid pYC166 that contain corresponding mutations. Derivatives of YC256 were named as YC256(M1), YC256(M2), YC256(M3, 4), YC256(Δ2-4), and YC256(RS2-4). The corresponding operator mutations in those derivative strains were described in Figure 2A.

3) Construction of YC285 and its derivatives

To construct YC285, we applied a method similar to long-flanking PCR mutagenesis (Wach, 1996). In the first round PCR reaction, a 1.2-kb DNA fragment starting from the middle of the *yqhH* gene to the downstream of *sinR* was amplified by PCR using primers *yqhH*-F1 and *yqhH*-R1 and 3610 chromosomal DNA as the template. A second DNA fragment starting from the end of the *tasA* gene to the middle of *tasA* was also amplified by PCR using primers *tasA*-F1 and *tasA*-R1. These two PCR products were gel-purified and used as primers in the second round PCR reaction, in which the *kan* drug

resistance gene from linealized plasmid pAH49 was used as the template. The products from the second round PCR reaction were introduced into PY79 by transformation and selected for kanamycin resistance. The resulting transformants contained a *kan* gene inserted to the integenic region between *sinR* and *tasA*. The DNA fragment containing the *kan* gene and the flanking sequences were then introduced into 3610 by transduction. The resulting strain was named YC285. YC285 is otherwise the same as 3610 except for the *kan* gene insertion.

To construct derivatives of YC285 that contained various operator mutations and deletions, we applied a slightly different strategy. First, in the first round PCR reaction, operator mutations or deletions were generated by using the method that was described previously and incorporated into the first PCR products starting from the middle of *yqhH* to the end of *sinR*. After the second round PCR reaction, the PCR products were introduced by transformation into a PY79 derivative mutant for Δ*sinI* Δ*sinR*. The resulting transformants were verified for the *kan* gene insertion between *sinR* and *tasA* and introduction of operator mutations or deletions by DNA sequencing. The DNA fragment containing *kan* and operator mutations was then introduced into 3610 by transduction.

To construct strains YC716 and YC717, the plasmid pYC213, which contains the reporter *amyE*::P_{yqxM}-*gfp-LCN*, was first introduced into PY79 by transformation. The DNA containing the reporter fusion, as well as the DNA containing the *epsH* null mutation (Δ*epsH*, from RL3852), was introduced into

both YC285 and YC285(Δ2-4) by transduction, resulting in strains YC716 and YC717, respectively.

4) Construction of Δ*sinI sinR* complementation strains

To construct Δ*sinI sinR* complementation strains at *amyE*(28°), *ylnF*(140°), *bkdB*(213°), and *ywrK*(317°) loci on the chromosome, the plasmid pYC191, which contains *yqhHG sinI sinR* fragment flanked by *amyE* fragments was first introduced by transformation into AHB277, AHB289, and AHB290 (Table S1), respectively. The transformants were selected for Cm^R and $Spec^S$ on the selective plates. In those transformants, *amyE*::*yqhHG sinI sinR* (from pYC191) was integrated at the *amyE* site in *ylnF*(140°), *bkdB*(213°), and *ywrK*(317°) loci, instead of at the native *amyE* locus. *amyE*:: *yqhHG sinI sinR* fragments were then introduced to a Δ*sinI sinR* strain of 3610 by SSP1-phage mediated transduction.

To construct a strain with two copies of *yqhHG sinI sinR* (YC562), one at the native locus, and the other integrated at an origin-distal site *bkdB*(213°), the *amyE*::*yqhHG sinI sinR* fragment integrated at *bkdB*(213°) that was constructed was simply introduced into 3610.

5) Construction of YC264 and YC553

Strain YC264 contains a spectinomycin resistance gene inserted between the 3' end of the *yqhG* gene and the promoter region of *sinI*. To construct YC264, a DNA fragment (~900-bp) starting from the 5' end of *yqhG* to the stop codon of

the *yqhG* gene was amplified by PCR using 3610 chromosomal DNA as template and primers P_{sinI}-KO-P1 and P_{sinI}-KO-P2. Similarly, a second DNA fragment (~900-bp) starting from the stop codon of the *yqhG* gene to the end of *sinR* was amplified by PCR using primers P_{sinI}-KO-P3 and P_{sinI}-KO-P4. PCR products were gel-purified. The first PCR products were cloned into the XhoI site of pAH54 (gift of Camp A), and the second PCR products were cloned sequentially into the BamHI and SacI sites of pAH54. The resulting plasmid (pYC171) contains the *spec* gene flanked by the above two DNA fragments. The plasmid was then introduced into PY79 by transformation. The *spec* resistance gene containing DNA fragment was then introduced into 3610 by transduction.

Strain YC553 was constructed very similarly to that of YC264. A few differences were described below. First, the first PCR product starts from 5' upstream of the *yqhH* promoter to the start codon of the *yqhH* gene, and was amplified by PCR using primers P_{yqhHG}-KO-P1 and P_{yqhHG}-KO-P2, the second PCR products starts from the start codon of *yqhH* to an internal site of *yqhH*. These two PCR products were cloned into pAH54 sequentially and generated a recombinant plasmid pYC202. The rest of the steps were the same as described above.

6) Construction of YC718 and YC719

To construct YC718 and YC719, the strain RL3618 (ref) in which the *spo0A* gene was replaced by an IPTG-inducible copy of *spo0A-sad67* (*spo0A*Ω$P_{hyspank}$-*spo0A-sad67*) was infected with SSP1 phage. The *sad67*

construct, as well as the reporter fusion *lacA*::P_{yqxM}-*yfp* (made from DL841, a gift of Lopez, D), was then introduced into YC285(Δ2-4) and a derivative of YC285(Δ2-4), in which the *yqhHG sinI sinR* fragment was transplanted to the origin-proximal *amyE* locus by transduction, resulting in strains YC718 and YC719.

7) Construction of YC734

Strain YC734 was constructed as follows. The recombinant plasmid pYC237 was first introduced into PY79 by transformation. The DNA containing the reporter fusion was then introduced into ALM91 (gift of McLoon A), a 3610 derivative that contains a luciferase reporter (Stewart & Williams, 1992) fused to the promoter of the *yqxM* operon (P_{yqxM}-*lux*) by transduction, resulting in strain YC734. The construction of the *luxABCDE* reporter was described previously (Schmalisch et al, 2010).

Fluorescent microscopic analysis of YC716 and YC717

YC716 and YC717 cells were grown in MSgg medium to stationary phase. 1-ml cells were harvested, washed twice with PBS buffer, and suspended in 50-μl of PBS buffer. Samples were mixed with FM4-64, and 3-μl of cells were applied to the center of an agar-coated microscopy slide. Cover slides were pretreated with poly-L-lysine (Sigma). Samples were analyzed using 100x oil merged lens on an Olympus workstation BX61. Images were taken using an

automated software program SimplePCI and analyzed with programs MetaMorph (Universal Imaging Corporation) and Image J (http://rsbweb.nih.gov/ij/).

Copy number control

1 A model for matrix gene expression

We consider the activity of a matrix promoter as some function $f(I, R)$ of SinI and SinR concentration. The SinR population is divided into two pools: $[IR]$ is the part bound by SinI and held inactive, and R^* is the free SinR that regulates target promoters. We denote the total SinR population by R, so that $R = [IR] + R*$.

As SinR regulation of target promoters is cooperative, it is reasonable to model the activity of a regulated promoter as a Hill function in R^*. We will for the moment leave the precise mechanism of SinI titration of SinR unspecified, instead assuming just that $[IR] = Rg(I)$ for some decreasing function g of SinI concentration. These two assumptions yield the following form for f (with normalized units of activity):

$$f(I, R) = \frac{K^n}{K^n + (Rg(I))^n},$$

where K is the apparent dissociation constant and n is the Hill coefficient.

2 Approximating fold change

We are interested in the relative change in promoter activity when the copy number of SinI and SinR is altered. If copy number increases, we assume each protein increases by some factor $c > 1$. An approximation of the change in promoter activity then follows by Taylor expansion:

$$\begin{aligned} & f(cI, cR) \approx f(I, R) + \nabla f(I, R) \cdot (cI - I, cR - R) \\ \Rightarrow \quad & \frac{f(cI, cI)}{f(I, R)} \approx 1 + (c-1)\left(\frac{I}{f(I,R)}\frac{\partial f}{\partial I}(I, R) + \frac{R}{f(I,R)}\frac{\partial f}{\partial R}(I, R)\right) \\ & \qquad = 1 + (c-1)(S_I(I, R) + S_R(I, R)), \end{aligned} \tag{1}$$

where we have recognized the logarithmic sensitivities $S_I = \frac{\partial \ln f}{\partial \ln I}$ and $S_R = \frac{\partial \ln f}{\partial \ln R}$.

In the case of the model described in the previous section, it can be shown by direct computation that

$$S_I = S_R \cdot \left(1 + \frac{\partial \ln g}{\partial \ln A}\right).$$

Christening $H_R := S_R$ and $H_I := \frac{\partial \ln g}{\partial \ln I}$, we recognize that each of these is negative. Plugging $|H_R| = -H_R$ and $|H_I| = -H_I$ into (1) and picking $c = 2$ yields the equation from the main paper:

$$\boxed{\frac{f(2I, 2R)}{f(I, R)} \approx 1 + |H_R|(|H_I| - 1)} \tag{2}$$

Having shown the general mechanism, we now specialize slightly. The requirement that activity of SinR-regulated promoters decreases in response to an increase in copy number implies that $|H_R|(|H_I| - 1) < 0$, which requires that $|H_I| < 1$. As it is known that SinI and SinR bind 1:1, we approximate the dynamics of the complex as:

$$\frac{d[IR]}{dt} = k_1[I][R^*] - k_{-1}[IR].$$

At steady state, together with the condition that $[IR] + R^* = R$, this equation shows that

$$[IR] = [R]\frac{[I]}{K_1 + [I]} \qquad \Rightarrow \qquad [R^*] = [R]\frac{K_1}{K_1 + [I]}.$$

Thus the free SinR concentration is given by the total SinR concentration multiplied against a Michaelis-Menten function. (In terms of the notation used previously, $g(I) = \frac{K_1}{K_1 + I}$.) The logarithmic sensitivity of Hill functions varies between 0 and the Hill coefficient, so this simple mechanism, where the Hill coefficient is 1, obviously satisfies the condition $|H_I| < 1$.

3 A note on the general case

It is clear that the derivations here have nothing to do with SinI or SinR in particular. If we have a gene regulated by a number of activators $A_1, \ldots, A_n$ and repressors $R_1, \ldots, R_n$ that undergo changes in copy number c_{A_i} and c_{R_i}, then it follows from the calculations above that

$$\frac{f(c_{A_1}A_1, \ldots, c_{A_n}A_n, c_{R_1}R_1, \ldots, c_{R_m}R_m)}{f(A_1, \ldots, A_n, R_1, \ldots, R_n)} \approx 1 + \sum_{i=1}^{n}(c_{A_i} - 1)\left|\frac{\partial \ln f}{\partial \ln A_i}\right| - \sum_{i=1}^{m}(c_{R_i} - 1)\left|\frac{\partial \ln f}{\partial \ln R_i}\right|.$$

Thus for a general promoter, the approximate fold change in activity is set by the copy number increase of a given regulator multiplied by the sensitivity with respect to that regulator, where activators cause increases and repressors cause decreases. As in the SinI-SinR case, larger changes follow from high sensitivity, which suggests that promoters where regulators bind with a high degree of cooperativity may be most likely to exhibit copy number control.

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