Supplementary Information

Nutritional Regulation of the Sae Two-Component System by CodY in *Staphylococcus aureus*

Kevin D. Mlynek, William E. Sause, Derek E. Moormeier, Marat R. Sadykov, Kurt R. Hill,

Kenneth W. Bayles, Victor J. Torres, and Shaun R. Brinsmade*

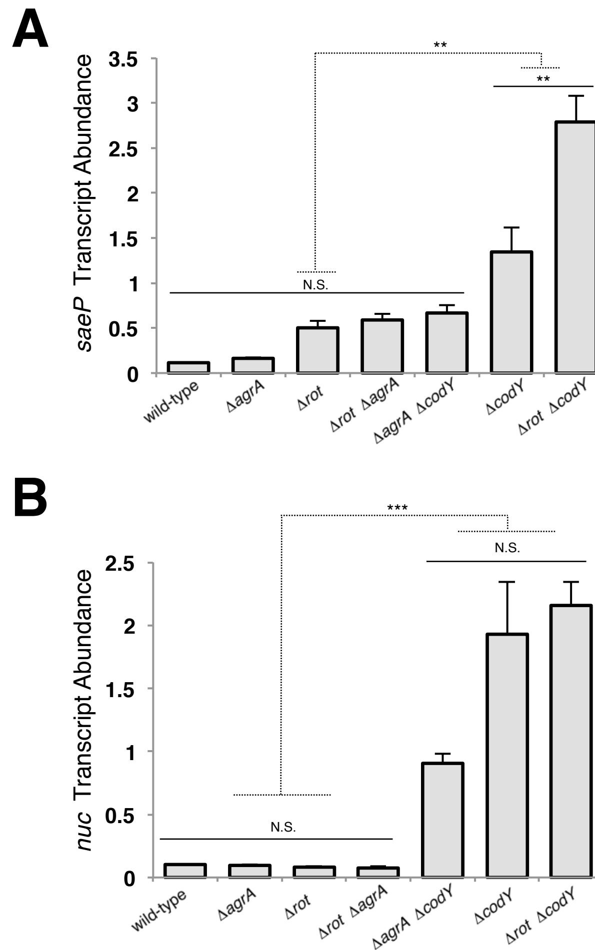

Figure S1: CodY controls SaeR targets indirectly via *agr* and Rot in UAMS-1. Mean transcript abundances of (A) *saeP* and (B) *nuc* are shown for a suite of *agrA* mutants during exponential growth in TSB using qRT-PCR. Transcripts were normalized to *rpoC* transcript. Statistical significance for each condition was accessed using one-way ANOVA with Tukey's multiple comparison post test (**$p < 0.01$, ***$p < 0.001$). Error bars represent the standard error of the mean from at least two independent experiments. Error bars are plotted for all data; occasionally the error bars are too small to see.

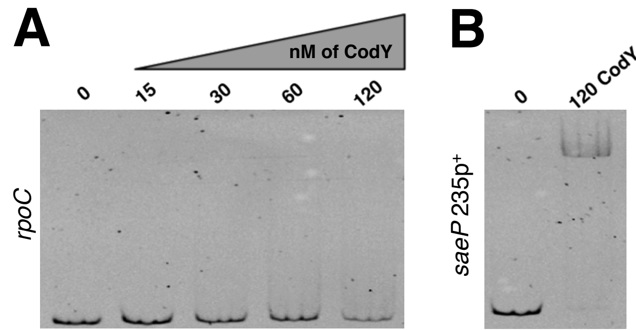

Figure S2: CodY is unable to bind a DNA fragment lacking a definable binding motif. (A) A 236-bp DNA fragment amplified from an internal portion of the *rpoC* gene (not differentially regulated by CodY) was incubated with increasing concentrations of CodY. (B) The *saeP235p*$^{+}$ fragment was used as a positive control on the same gel. The concentration (nM of monomer) used in each reaction is indicated above each lane. Gel images are representative of multiple experiments.

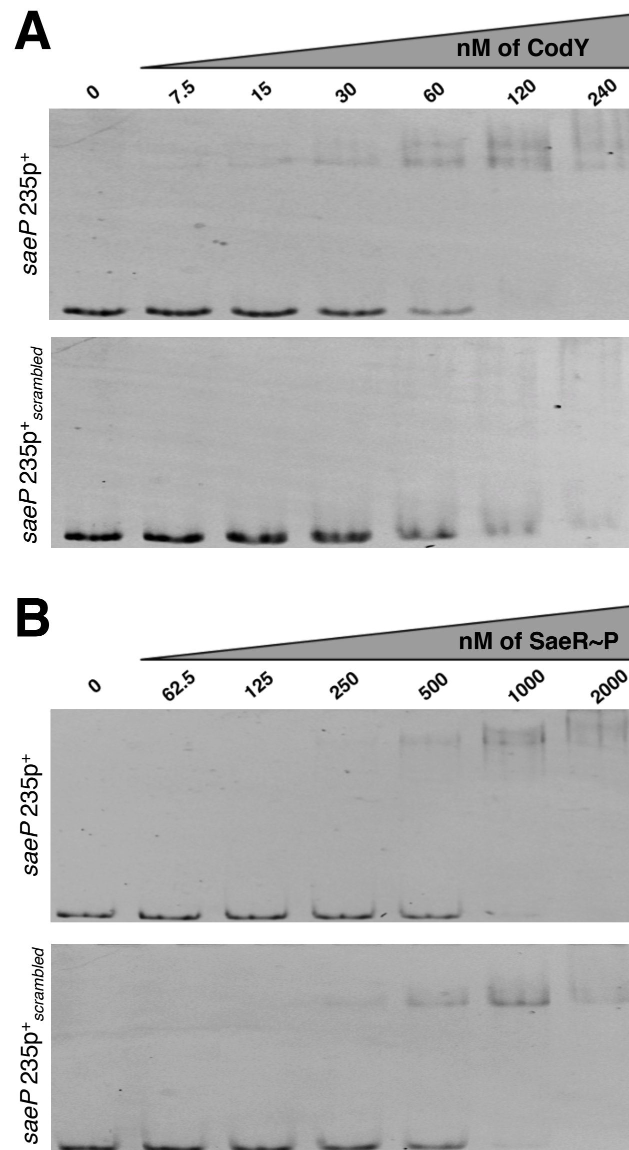

Figure S3: Scrambling the CodY binding site in the SaeP upstream region significantly reduces interaction with CodY protein, but not the interaction with SaeR~P. The wild-type *saeP235p+* fragment or a fragment with a scrambled CodY-binding site (*saeP235p$_{scrambled}$*) was incubated with increasing concentrations of CodY (A) or SaeR~P (B). The concentration (nM of monomer) used in each reaction is indicated above each lane.

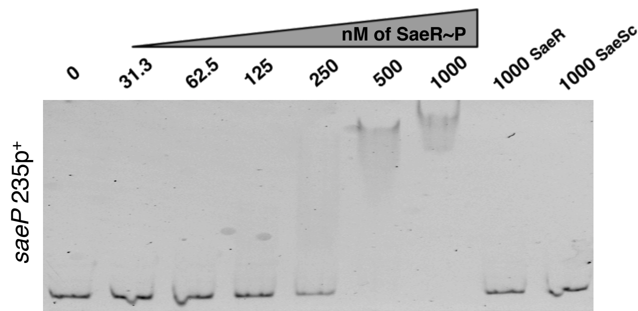

Figure S4: SaeR requires phosphorylation to bind the *saeP235p⁺* fragment. The wild-type *saeP235p⁺* fragment was incubated with increasing concentrations of SaeR~P. SaeR requires phosphorylation by SaeS to interact with DNA (1); control binding reactions containing only SaeR or SaeSC were set up in parallel to confirm interaction only with SaeR~P. The concentration (nM of monomer) used in each reaction is indicated above each lane.

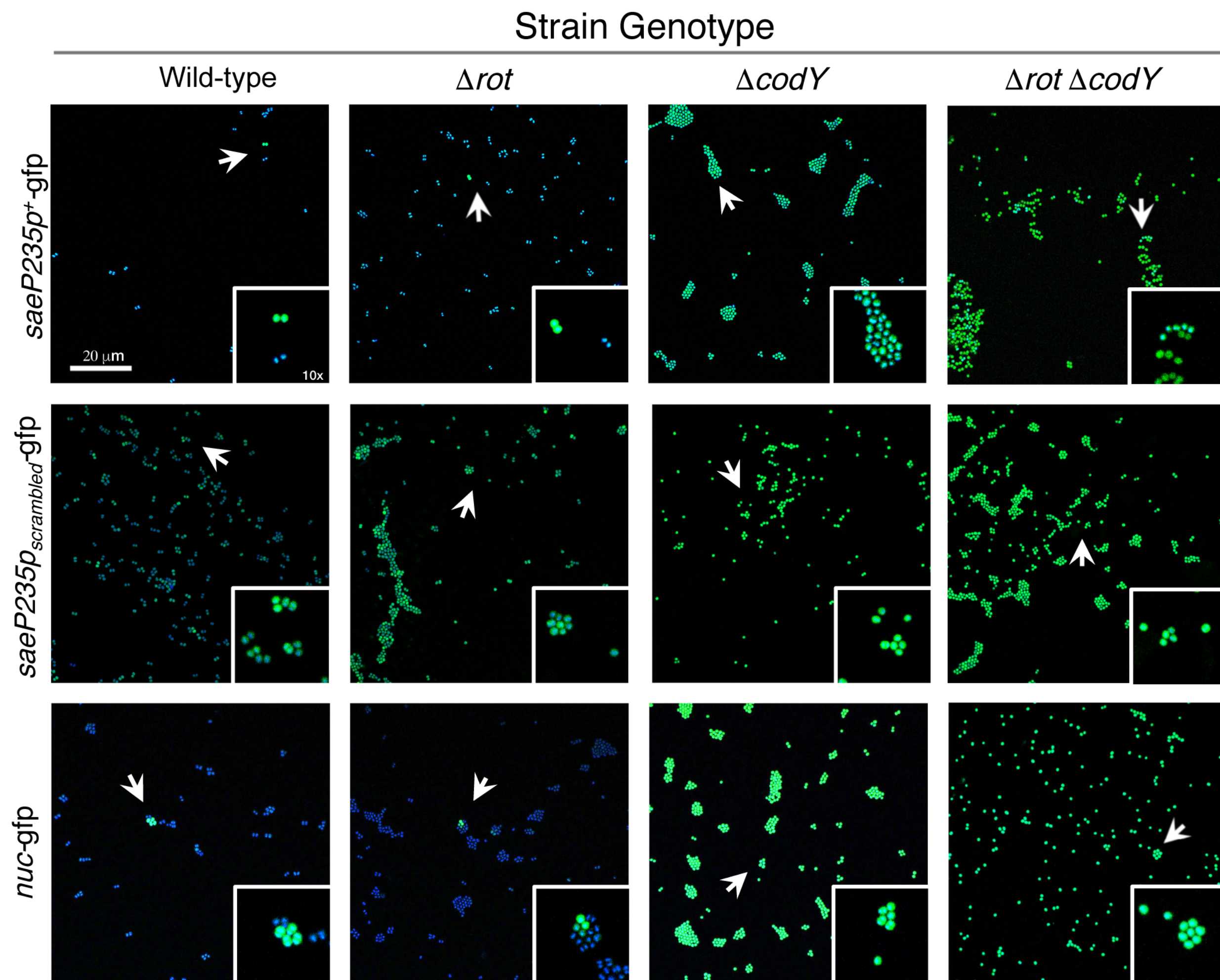

Figure S5: Stochastic expression of SaeR targets is alleviated when *codY* is knocked out. UAMS-1 cells harboring *gfp* reporter fusions were grown to exponential phase, fixed, and examined using fluorescent microscopy. GFP fluorescence, indicating promoter activity, is false-colored green. DAPI staining is used to visualize all cells and is false-colored blue. White arrows indicate regions magnified in inset images (10x). Images are representative of at least two independent experiments.

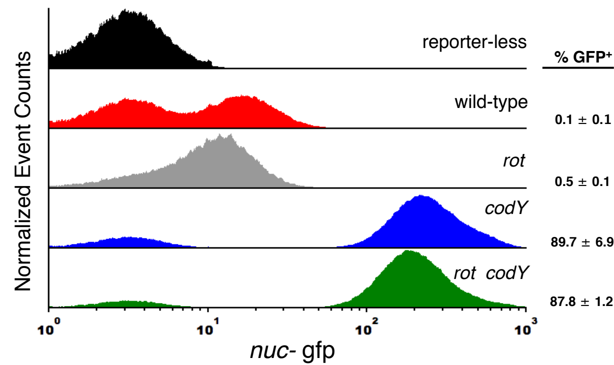

Figure S6: The frequency of GFP$^+$ cells expressing the *nuc-gfp* fusion increases in the absence of CodY mediated repression. Flow cytometry analysis of UAMS-1 cells carrying a *nuc-gfp* reporter fusion fixed in 4% PFA during exponential growth. Event counts were normalized after analyzing at least 30,000 cells. Percent of GFP$^+$ cells was determined using events that exceeded the maximal fluorescence of reporter-less cells. Histograms displayed are representative of two independent experiments.

Table S1. Bacterial strains used in this study.

***S. aureus* strains**	**Relevant genotype or description**	**Reference or source[a]**
RN4220	restriction deficient, highly transformable	(2)
PM783	Δ*rot*::Tn*917*	R. Procter
SRB337	USA200 MSSA UAMS-1	(3)
SRB372	SRB337 Δ*codY::ermC*	(4)
SRB872	SRB337 Δ*rot*::Tn*917*	
SRB899	SRB337 Δ*codY::ermC* Δ*rot*::Tn*917*	
SRB548	SRB337 Δ*agrA*	
SRB549	SRB337 Δ*agrA* Δ*codY::ermC*	
SRB898	SRB337 Δ*agrA* Δrot::Tn*917*	
SRB899	SRB337 Δ*codY::ermC* Δ*rot*::Tn*917*	
SRB687	USA300 LAC CA-MRSA Em^S	A. Horswill
SRB746	SRB687 Δ*codY::ermC*	(4)
SRB858	SRB687 Δ*rot*::Tn*917*	
SRB865	SRB687 Δ*codY::ermC* Δ*rot*::Tn*917*	
SRB1008	SRB337/pKM11 *bla cat saeP235p⁺-gfp*	
SRB1009	SRB372/pKM11	
SRB1042	SRB872/pKM11	
SRB1043	SRB899/pKM11	
SRB1056	SRB337/pMRS-nuc *bla cat nuc-gfp*	
SRB1057	SRB372/pMRS-nuc	
SRB1159	SRB872/pMRS-nuc	
SRB1160	SRB899/pMRS-nuc	
SRB1162	SRB337/pKM14 *bla cat* $saeP235p_{scrambled}$*-gfp*	
SRB1163	SRB372/pKM14	
SRB1164	SRB872/pKM14	
SRB1165	SRB899/pKM14	
***E. coli* strains**		
BL21 Star (λDE3)	F^- *ompT* $hsdS_B$ (r_B^-, m_B^-) *gal dcm rne131* (λDE3)	Novagen
NEB-5α	*fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	New England Biolabs

[a] Unless noted otherwise strains were constructed during the course of this study.

Table S2: Primers used in this study[a].

Primer	Sequence (5' to 3')[b,c]	Purpose
oKM026	CGGCGCATGCAAGTGTTTTATAGTGATAAC	Construction of pKM11 (*$saeP235p^{+}$-gfp*)
oSRB484	GGGCATGCTAACTCCTCATTTCTTCAATTTGAT	Construction of pKM11 (*$saeP235p^{+}$-gfp*)
oKM062	*GGCTACTTAAGTCCCTGCGTAAGGA*GAAAATAATTGTCTGATTTAAATAAATAGG	Construction of pKM15 (*$saeP235p_{scrambled}$-gfp*)
oKM063	*TCCTTACGCAGGGACTTAAGTAGCC*ATTAGTTAAGCGATATTTAAACGAAG	Construction of pKM15 (*$saeP235p_{scrambled}$-gfp*)
oKM025	6-FAM/GGGCATGCTAACTCCTCATTTCT	5'FAM labeled oSRB484 for EMSA
oKM031	6-FAM/CGGCGCATGCAAGTGTTTTATAG	5'FAM labeled oKM026 for EMSA
oKM027	6-FAM/CTTTCACGACGTACTTTAGA	5'FAM labeled oSRB240 for EMSA
oKM048	5-MAXN/GGGCATGCTAACTCCTCATTTCTTCAATTT	5'MAX labeled oKM025 for DFACE
oDS001	CGAAAGAACAATACGCAAAGAGG	qRT-PCR of *nuc*
oDS002	TGCATTTGCTGAGCTACTTAGA	qRT-PCR of *nuc*
oDS018	CGGTGAAACTGTTGAAGGTAAAG	qRT-PCR of *saeP*
oDS019	CGTAGTCAACCATTGCGATTTC	qRT-PCR of *saeP*
oSRB239	GGATTGGCTTCACCTGAA AA	qRT-PCR of *rpoC*
oSRB240	CTTTCACGACGTACTTTAGA	qRT-PCR of *rpoC*
oKM1	GCATTGAGCTCAAAGGAGAAAAATTCATGAGC	Construction of SaCodY-His$_6$ in pBAD30
oSRB410	ATGATGATGGCCCTGAAAATACAGGTTTTCCTTACTTTTTTCTAATTCATCT	Construction of SaCodY-His$_6$ in pBAD30
oSRB411	AGCTTGCATGCTTAATGATGATGATGATGATGGCCCTGAAAATACAGGTTTTC	Construction of SaCodY-His$_6$ in pBAD30

[a] All primers used in this study were designed using UAMS-1 as the reference genome.
[b] Underline denotes the presence of a restriction site for cloning into pMRSI.
[c] Italics denotes complementary sequences for use in overlapping PCR.

Table S3: Plasmids used in this study.

Plasmid	Relevant Genotype	Reference or source[a]
pMRSI	sGFP-sDsRed double reporter shuttle vector (Ap^R, Cm^R)	(4)
pMRSI-nuc	pMRSI with 382 bp upstream of *nuc* fused to sGFP (*nuc-gfp*) (Ap^R, Cm^R)	(4)
pKM11	pMRSI with 235 bp upstream of *saeP* fused to sGFP (*saeP*235-*gfp*) (Ap^R, Cm^R)	
pKM14	pKM11 with scrambled CodY binding motif (*saeP*$235_{scrambled}$-*gfp*) (Ap^R, Cm^R)	
pSRB81	Overproduction plasmid for *Sa*CodY-His_6 protein (Ap^R)	
pET284	Overproduction plasmid for *Sa*SaeR-His_6 protein (Ap^R)	(1)
pMCS619	Overproduction plasmid for *Sa*SaeSc-His_6 protein (Ap^R)	(1)
pRK1037	Produces TVMV protease for controlled intracellular processing of fusion proteins (Km^R)	Science Reagents, Inc.
pBAD30	Plasmid with P_{araBAD} for arabinose-inducible expression (Ap^R)	(5)

[a] Unless otherwise noted all plasmids were engineered during this study.

References

1. **Sun F, Li C, Jeong D, Sohn C, He C, Bae T.** 2010. In the *Staphylococcus aureus* two-component system *sae*, the response regulator SaeR binds to a direct repeat sequence and DNA binding requires phosphorylation by the sensor kinase SaeS. J Bacteriol **192:**2111-2127.

2. **Schenk S, Laddaga RA.** 1992. Improved method for electroporation of *Staphylococcus aureus*. FEMS Microbiol Lett **73:**133-138.

3. **Gillaspy AF, Hickmon SG, Skinner RA, Thomas JR, Nelson CL, Smeltzer MS.** 1995. Role of the accessory gene regulator (*agr*) in pathogenesis of staphylococcal osteomyelitis. Infect Immun **63:**3373-3380.

4. **Waters NR, Samuels DJ, Behera RK, Livny J, Rhee KY, Sadykov MR, Brinsmade SR.** 2016. A Spectrum of CodY Activities Drives Metabolic Reorganization and Virulence Gene Expression in *Staphylococcus aureus*. Mol Microbiol **101:**495-514.

5. **Guzman L-M, Belin D, Carson MJ, Beckwith J.** 1995. Tight regulation, modulation, and high-level expression by vectors containing arabinose P_{BAD} promoter. J Bacteriol **177:**4121-4130.